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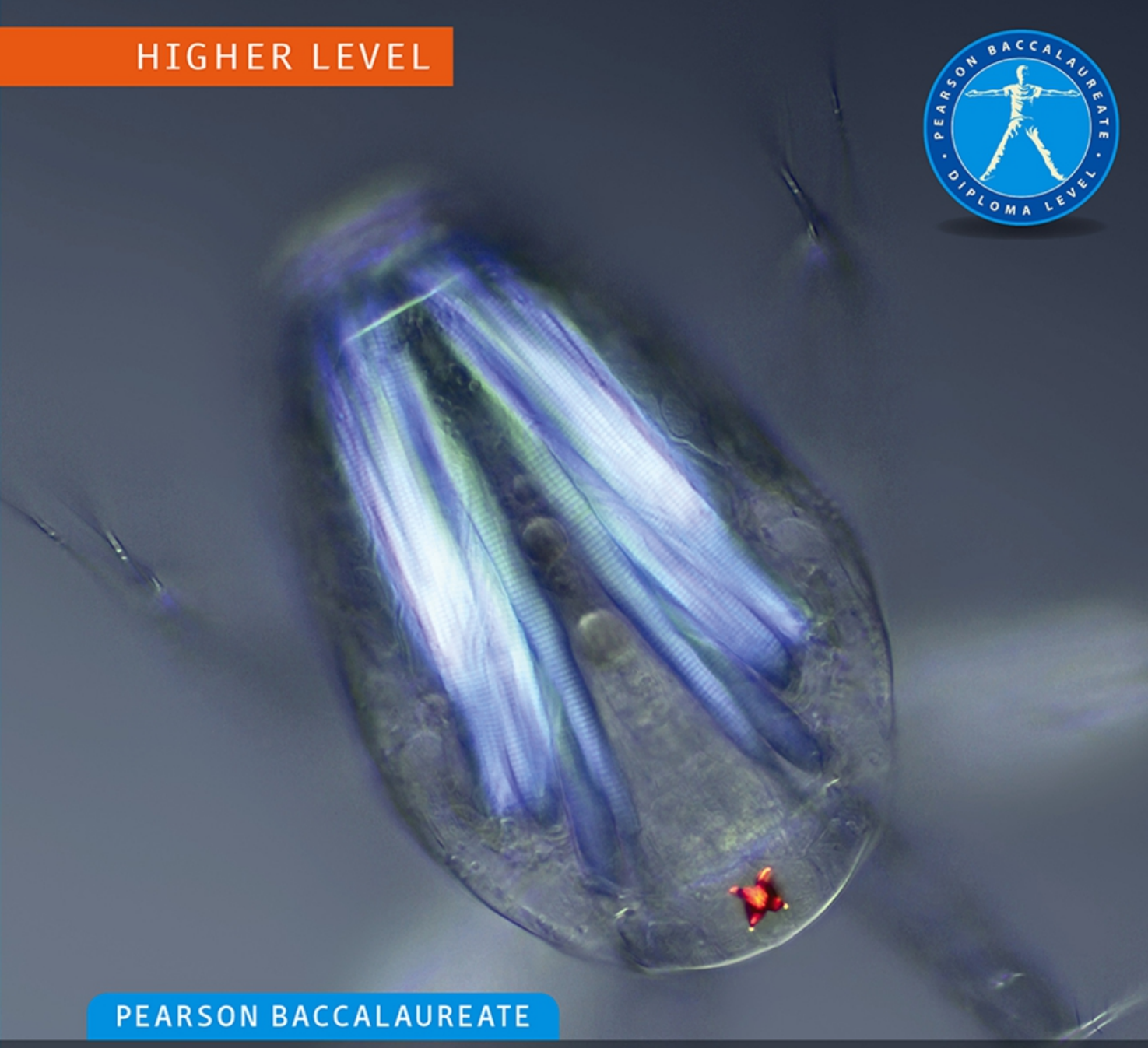

PEARSON BACCALAUREATE

HIGHER LEVEL

Biology

2nd Edition

ALAN DAMON • RANDY McGONEGAL • PATRICIA TOSTO • WILLIAM WARD

Supporting every learner across the IB continuum

ALWAYS LEARNING

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HIGHER LEVEL

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Dedications

To my father, the late Dr William A. Damon, a man of principle, an intellectual giant, and a dear friend.

Alan Damon

To my children and grandchildren. You are my future even when I am gone.

Randy McGonegal

I dedicate this book to my husband, who has been my editor and constant support.

Pat Tosto

I dedicate this book to the most important people in my life, my family. You have allowed me to be more than I ever could have been without you.

Bill Ward

Contents

Contents

Introduction

Authors' introduction to the second edition

Welcome to your study of International Baccalaureate (IB) Higher Level (HL) biology. This book is the second edition of the market-leading Pearson Baccalaureate HL biology book, first published in 2007. It has been completely rewritten to match the specifications of the new IB biology curriculum, and gives thorough coverage of the entire course content. While there is much new and updated material, we have kept and refined the features that made the first edition so successful. Our personal experience and intimate knowledge of the entire IB biology experience, through teaching and examining, curriculum review, moderating internal assessment, and leading workshops for teachers in different continents, has given us a unique understanding of your needs in this course. We are delighted to share our enthusiasm for learning biology in the IB programme with you!

Content

The book covers the three parts of the IB syllabus: the core, the AHL (additional higher level) material, and the options, of which you will study one. Each chapter in the book corresponds to a topic or option in the IB guide, in the same sequence. The sequence of sub-topics within each chapter is given in the contents page.

Each chapter starts with a list of the Essential ideas from the IB biology guide, which summarize the focus of each sub-topic.

Essential ideas

3.2 Chromosomes carry genes in a linear sequence that is shared by members of a species.

This is followed by an introduction, which gives the context of the topic and how it relates to your previous knowledge. The relevant sections from the IB biology guide for each sub-topic are then given as boxes showing Understandings and Applications and skills, with notes for Guidance shown in italics where they help interpret the syllabus.

Understandings:

- Prokaryotes have one chromosome consisting of a circular DNA molecule.
- Some prokaryotes also have plasmids but eukaryotes do not.

Applications and skills:

- Application: Non-disjunction can cause Down syndrome and other chromosome abnormalities.
- Application: Studies showing age of parents influences chances of non-disjunction.
- Skill: Drawing diagrams to show the stages of meiosis resulting in the formation of four haploid cells.

Guidance

- *Preparation of microscope slides showing meiosis is challenging and permanent slides should be available in case no cells in meiosis are visible in temporary mounts.*

The text covers the course content using plain language, with all key scientific terms explained in the eBook glossary.

We have been careful also to apply the same terminology you will see in IB examinations in all worked examples and questions.

The nature of science

Throughout the course you are encouraged to think about the nature of scientific knowledge and the scientific process as it applies to biology. Examples are given of the evolution of biological theories as new information is gained, the use of models to conceptualize our understanding, and the ways in which experimental work is enhanced by modern technologies. Ethical considerations, environmental impacts, the importance of objectivity, and the responsibilities regarding scientists' code of conduct are also considered here. The emphasis is on appreciating the broader conceptual themes in context. You should familiarize yourself with these examples to enrich your understanding of Biology. We have included at least one example in each sub-section, and hope you will come up with your own as you keep these ideas at the surface of your learning.

Key to information boxes

A popular feature of the book is the different coloured boxes interspersed throughout each chapter. These are used to enhance your learning as explained using the examples below.

Nature of science

This is an overarching theme in the course to promote concept-based learning. Throughout the book you should recognize some similar themes emerging across different topics. We hope they help you to develop your own skills in scientific literacy.

NATURE OF SCIENCE

Most, but not all, organisms assemble proteins from the same 20 amino acids. Virtually every reference concerning amino acids will tell you that there are 20 amino acids in nature. It is true that the universal genetic code (universal indicating that it is used in the vast majority of organisms on Earth) only encodes 20. But in nature there are frequently exceptions, and that includes things that are called 'universal'. If you include all known living organisms then there are 22 amino acids that are used to create polypeptides. In addition to the 20 amino acids whose structures are given in Figure 2.20, there are two additional amino acids called selenocysteine and pyrrolysine.

International-mindedness

The impact of the study of biology is global, and includes environmental, political, and socio-economic considerations. Examples of this are given to help you to see the importance of biology in an international context.

Even though the first accurate model of DNA was produced by James Watson (American) and Francis Crick (British) in 1953, many other scientists from around the world contributed pieces of information that were instrumental in developing the final model. Erwin Chargaff (Austrian) had determined that the numbers of adenine and thymine bases were equal, as were the numbers of cytosine and guanine bases. Rosalind Franklin (British) and Maurice Wilkins (born in New Zealand) had calculated the distance between the various molecules in DNA by X-ray crystallography.

Utilization

Applications of the topic through everyday examples are described here, as well as brief descriptions of related biological industries. This helps you to see the relevance and context of what you are learning.

Gene therapy is the process of taking a beneficial gene from a person who possesses it and putting it into a person who does not have it, but who needs it to stay healthy. The challenge is that it is very difficult to get the DNA into the sick person's cells. One way is to force the gene into the patient's cells using a virus to deliver it. Partly because of a lack of understanding of how to use viruses safely to deliver genes, the decision was made to stop all testing of gene therapy on human patients in the USA in 1999, when an 18-year-old patient died after a virus had been injected into his body. However, gene therapy trials are coming back, little by little, notably in helping blind children to regain their eyesight.

In the 1997 science fiction film *GATTACA*, one of the main characters brings a sample of cells to a walk-up window at an establishment that provides anonymous genome services. Within seconds, she gets a full printout and analysis of the genome she is interested in. One objective of science fiction as an art form is to warn society of what might happen in the future if we are not careful. This film raises questions about how far technology will lead us and whether or not we want to go in that direction. Our society will need to make some difficult decisions in the coming years concerning our genomes and who has access to the information contained within them.

Interesting fact

These give background information that will add to your wider knowledge of the topic and make links with other topics and subjects. Aspects such as historic notes on the life of scientists and origins of names are included here.

Laboratory work

These indicate links to ideas for lab work and experiments that will support your learning in the course, and help you prepare for the Internal Assessment. Some specific experimental work is compulsory, and further details of this are in the eBook.

Investigating the factors that affect the rooting of stem cuttings

Design an experiment to assess one factor affecting the rooting of stem cuttings. The basic idea is to cut a few centimetres of stem from a healthy plant and place it into an appropriate medium either sticking up or having it lying flat. Typical plants to try are impatiens, begonias, jade, or African violet.

Who should decide how fast and how far humans should go with our study of DNA and the technology that is rapidly emerging?

TOK

These stimulate thought and consideration of knowledge issues as they arise in context. Each box contains open questions to help trigger critical thinking and discussion.

Key fact

These key facts are drawn out of the main text and highlighted in bold. This will help you to identify the core learning points within each section. They also act as a quick summary for review.

There are three main sources for variation in a population:

- **mutations in DNA**
- **meiosis**
- **sexual reproduction.**

Whenever a definition is given for a major concept in biology, in this instance the term 'gene', be sure to memorize its definition word for word. Such definitions have been phrased carefully so that all the important details are included.

Hints for success

These give hints on how to approach questions, and suggest approaches that examiners like to see. They also identify common pitfalls in understanding, and omissions made in answering questions.

Challenge yourself

These boxes contain open questions that encourage you to think about the topic in more depth, or to make detailed connections with other topics. They are designed to be challenging and to make you think.

CHALLENGE YOURSELF

8 Use the symbols mentioned above to represent all the possible nucleotides of DNA.

eBook

In the eBook you will find the following:

- Animations
- Videos
- Interactive glossary of scientific words used in the course
- Internal assessment advice
- Answers to all exercises in the book
- Worksheets
- Interactive quizzes

For more details about your eBook, see the following section.

Questions

There are three types of question in this book:

1 Worked example with Solution

These appear at intervals in the text and are used to illustrate the concepts covered. They are followed by the solution, which shows the thinking and the steps used in solving the problem.

Worked example

You are walking outside with a friend who is wearing a red and white shirt. Explain why the shirt appears to be red and white.

Solution

Sunlight is a mixture of all of the wavelengths (colours) of visible light. When sunlight strikes the red pigments in the shirt, the blue and the green wavelengths of light are absorbed, but the red wavelengths are reflected. Thus, our eyes see red. When sunlight strikes the white areas of the shirt, all the wavelengths of light are reflected and our eyes and brain interpret the mixture as white.

2 Exercises

These questions are found throughout the text. They allow you to apply your knowledge and test your understanding of what you have just been reading. The answers to these are given in the eBook at the end of each chapter.

Exercises

25 Explain why a blue object appears to be blue to the human eye.

26 Explain why black surfaces (like tarmacadam and asphalt) get much hotter in sunlight than lighter surfaces (like stone and concrete).

27 Plants produce sugars by photosynthesis. What do plants do with the sugars after that?

28 Why do most plants produce an excess of sugars in some months of the year?

3 Practice questions

These questions are found at the end of each chapter. They are mostly taken from previous years' IB examination papers. The markschemes used by examiners when marking these questions are given in the eBook, at the end of each chapter.

Practice questions

1 Draw the basic structure of an amino acid, and label the groups that are used in peptide bond formation.

(*Total 4 marks*)

Answers

Full answers to all exercises and practice questions can be found in the eBook.

Hotlink boxes can be found at the end of each chapter, indicating that there are weblinks available for further study. To access these links go to www.pearsonhotlinks.com and enter the ISBN or title of this book. Here you can find links to animations, simulations, movie clips and related background material, which can help to deepen your interest and understanding of the topic.

We truly hope that this book and the accompanying online resources help you enjoy this fascinating subject of IB Higher Level biology. We wish you success in your studies.

Alan Damon, Randy McGonegal, Pat Tosto, Bill Ward

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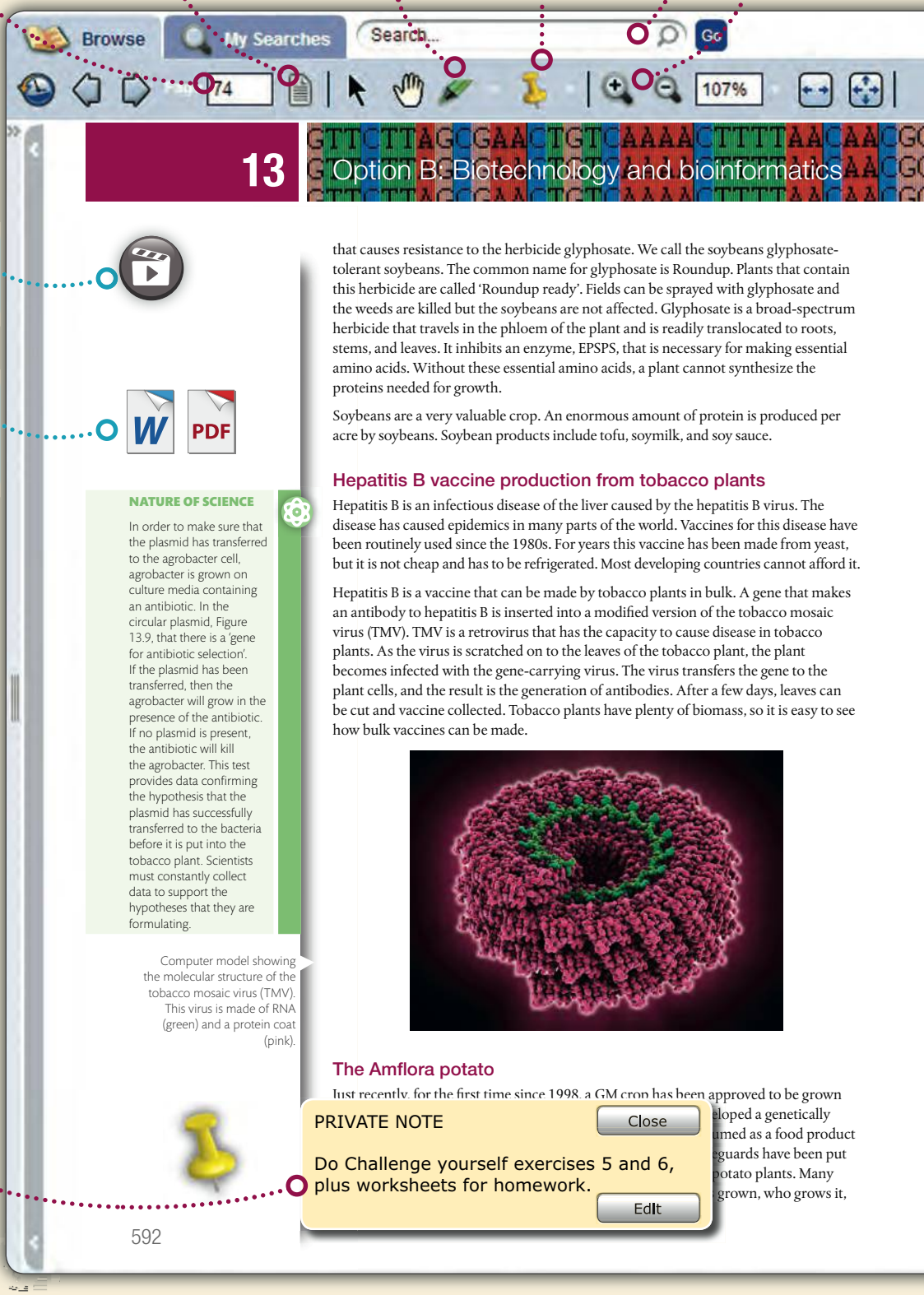

that causes resistance to the herbicide glyphosate. We call the soybeans glyphosate-tolerant soybeans. The common name for glyphosate is Roundup. Plants that contain this herbicide are called 'Roundup ready'. Fields can be sprayed with glyphosate and the weeds are killed but the soybeans are not affected. Glyphosate is a broad-spectrum herbicide that travels in the phloem of the plant and is readily translocated to roots, stems, and leaves. It inhibits an enzyme, EPSPS, that is necessary for making essential amino acids. Without these essential amino acids, a plant cannot synthesize the proteins needed for growth.

Soybeans are a very valuable crop. An enormous amount of protein is produced per acre by soybeans. Soybean products include tofu, soymilk, and soy sauce.

Hepatitis B vaccine production from tobacco plants

Hepatitis B is an infectious disease of the liver caused by the hepatitis B virus. The disease has caused epidemics in many parts of the world. Vaccines for this disease have been routinely used since the 1980s. For years this vaccine has been made from yeast, but it is not cheap and has to be refrigerated. Most developing countries cannot afford it.

Hepatitis B is a vaccine that can be made by tobacco plants in bulk. A gene that makes an antibody to hepatitis B is inserted into a modified version of the tobacco mosaic virus (TMV). TMV is a retrovirus that has the capacity to cause disease in tobacco plants. As the virus is scratched on to the leaves of the tobacco plant, the plant becomes infected with the gene-carrying virus. The virus transfers the gene to the plant cells, and the result is the generation of antibodies. After a few days, leaves can be cut and vaccine collected. Tobacco plants have plenty of biomass, so it is easy to see how bulk vaccines can be made.

The Amflora potato

Just recently, for the first time since 1998, a GM crop has been approved to be grown
eloped a genetically
umed as a food product
guards have been put
otato plants. Many
grown, who grows it,

See the definitions of key terms in the glossary

Create a bookmark

Switch to whiteboard view

The potato is called the Amflora potato, and it is a breakthrough in production of amylopectin, a type of starch made by potatoes. Normally, potatoes produce 20% amylose and 80% amylopectin. The Amflora potato produces 100% amylopectin, which is a desirable product for industry. The gene in this potato that produces the 20% amylose has been turned off. Amflora starch is beneficial to the paper and adhesive industry. It gives printer paper a glossier look and makes concrete stick better to walls.

Amflora: a genetically optimized potato that produces only one starch component and is used for technical applications.

NATURE OF SCIENCE

Scientists must assess the risks and benefits associated with scientific research. Genetic modification of crops has many risks to be considered:

- the potential for herbicide-resistance genes to escape into the wild population
- unintended harm to other organisms, such as insect pollinators and amphibians
- reduced effectiveness of herbicides
- possible human health risks, for example some studies have found glyphosate in human urine.

Have there been allergic reactions to the new gene put into a plant?

Despite regulatory approval by the EU, on 16 January 2012 BASF announced that it is pulling its genetic engineering division out of Europe and stopping production of its GM Amflora potato for the European market. The reason cited was lack of acceptance of this technology by consumers, farmers, and politicians

NATURE OF SCIENCE

Are the risks worth it? Use the hotlinks at the end of this section to watch a movie called GMO/OMG premiering in New York City in September 2013.

CHALLENGE YOURSELF

Adoption rates of GR (glyphosate-resistant) soybeans and cotton in the USA are shown in Figure 13.10. This bar chart shows the percentage of crop adoption over a 10-year period. Look at the bar chart and answer the following questions.

5 Compare and contrast the data regarding the two plant species.

6 Suggest a reason that might explain the differences.

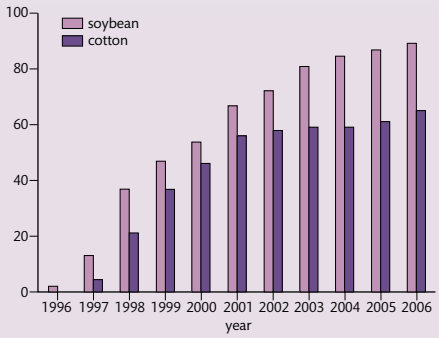

Figure 13.10 The percentage of soybean and cotton crop adoption over 10 years. Duke and Cerdeira 2007, Fig. 1

Animation
Select the icon to see a related animation

Quiz
Select the icon to take an interactive quiz to test your knowledge

Answers
Select the icon at the end of the chapter to view answers to exercises in this chapter

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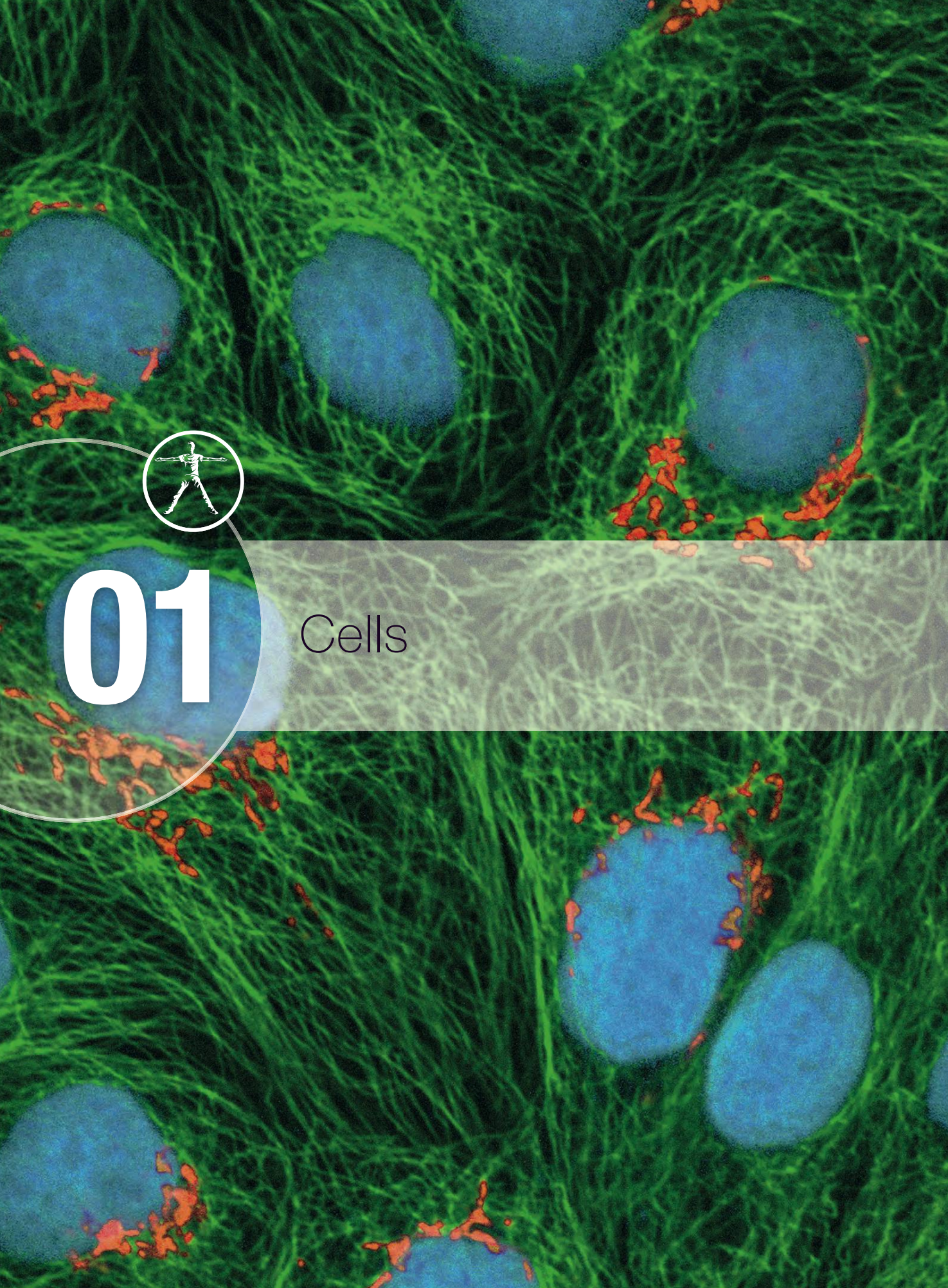


01 Cells

Essential ideas

1.1	The evolution of multicellular organisms allowed cell specialization and cell replacement.
1.2	Eukaryotes have a much more complex cell structure than prokaryotes.
1.3	The structure of biological membranes makes them fluid and dynamic.
1.4	Membranes control the composition of cells by active and passive transport.
1.5	There is an unbroken chain of life from the first cells on Earth to all cells in organisms alive today.
1.6	Cell division is essential but must be controlled.

HeLa cells were the first cells to be successfully cultured on a large scale and have been used extensively in biological research, including the development of the first polio vaccine.

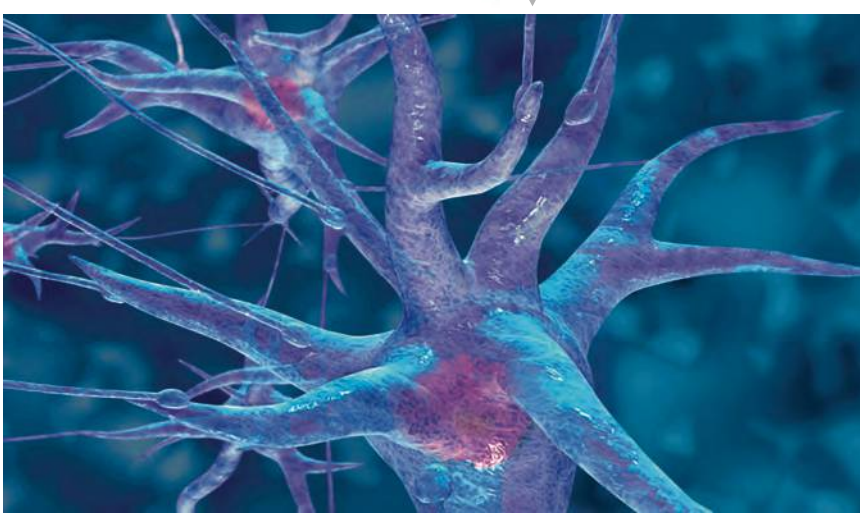
Cytology is the study of all aspects of a cell. As our understanding of the cell has increased, so has our ability to understand all forms of life, including diseases, that occur on Earth. However, there is still much work to be done in order to solve all the mysteries of the cell. Biological research laboratories all over the world are very active in this area.

Whether organisms are extremely small or extremely large, it is vital we understand their smallest functional units. These units are known as cells. Organisms range in size from a single cell to trillions of cells. To understand better all the organisms around us we must study their cells.

In this chapter, we will begin with a look at cell theory. After cell theory we will learn about the differences between prokaryotic and eukaryotic cells. A detailed explanation of cell parts and their functions will then follow. As much attention today is given to cancer, which seems to occur in most organisms and involves abnormal cell reproduction, we will focus on normal cell reproduction. Some time will also be spent on understanding how the most complex cells may have come into existence on our planet.

Look at the picture on the right. Human nerve cells (neurones) are essential to our lives. Because of these cells, we are able to acknowledge and respond to our surroundings. Neurones are usually very efficient but sometimes things go wrong. Can we gain a greater understanding and better treatment of conditions such as depression by learning more about how these cells function?

This is an artist's impression of human nerve cells.



NATURE OF SCIENCE

Looking for trends and discrepancies: although most organisms conform to cell theory, there are exceptions.

Ethical implications of research: research involving stem cells is growing in importance and raises ethical issues.

1.1 Cell theory, cell specialization, and cell replacement

Understandings:

- According to the cell theory, living organisms are composed of cells.
- Organisms consisting of only one cell carry out all functions of life in that cell.
- Surface area to volume ratio is important in the limitation of cell size.
- Multicellular organisms have properties that emerge from the interaction of their cellular components.
- Specialized tissues can develop by cell differentiation in multicellular organisms.
- Differentiation involves the expression of some genes and not others in a cell's genome.
- The capacity of stem cells to divide and differentiate along different pathways is necessary in embryonic development and also makes stem cells suitable for therapeutic uses.

Applications and skills:

- Application: Questioning the cell theory using atypical examples, including striated muscles, giant algae, and aseptate fungal hyphae.
- Application: Investigation of functions of life in *Paramecium* and one named photosynthetic unicellular organism.
- Application: Use of stem cells to treat Stargardt's disease and one other named condition.
- Application: Ethics of the therapeutic use of stem cells from specially created embryos, from the umbilical cord blood of a newborn baby and from an adult's own tissues.
- Skill: Use of a light microscope to investigate the structure of cells and tissues, with drawing of cells. Calculation of the magnification of drawings and the actual size of structures and ultrastructures shown in drawings or micrographs.

Guidance

- *Students are expected to be able to name and briefly explain these functions of life: nutrition, metabolism, growth, response, excretion, homeostasis, and reproduction.*
- Chlorella *or* Scenedesmus *are suitable photosynthetic unicells, but* Euglena *should be avoided as it can feed heterotrophically.*
- *Scale bars are useful as a way of indicating actual sizes in drawings and micrographs.*

Cell theory

It has taken several hundred years of research to formulate the cell theory that is used today. Many scientists have contributed to developing the three main principles of this theory. These three principles are:

1. all organisms are composed of one or more cells
2. cells are the smallest units of life
3. all cells come from pre-existing cells.

Cell theory has a very solid foundation largely because of the use of the microscope. Robert Hooke first described cells in 1665 after looking at cork with a self-built microscope. A few years later Antonie van Leeuwenhoek observed the first living cells and referred to them as 'animalcules', meaning little animals. In 1838, the botanist Matthias Schleiden stated that plants are made of 'independent, separate beings' called cells. One year later, Theodor Schwann made a similar statement about animals.

The second principle continues to gain support today, because so far no one has been able to find any living entity that is not made of at least one cell.

Some very famous scientists, such as Louis Pasteur in the 1880s, have performed experiments to support the third principle. After sterilizing chicken broth (soup) by

boiling it, Pasteur showed that living organisms would not 'spontaneously' reappear. Only after exposure to pre-existing cells was life able to re-establish itself in the sterilized chicken broth.

NATURE OF SCIENCE

As with most scientific theories, cell theory is not without areas of concern and problems. A key characteristic of a good scientist is a sceptical attitude towards theoretical claims. To overcome or validate this scepticism, evidence obtained by observation or experimentation is essential. Whenever possible in science, controlled experiments are needed to verify or refute theories. These experiments have a control group and a variable group(s). The groups are kept under similar conditions apart from the factor that is being tested or questioned. The factor being tested is referred to as the independent variable. The dependent factor is measured or described using quantitative or qualitative data. Relatively recent findings that have raised questions about cell theory include observations of striated muscle, giant algae, and aseptate fungal hyphae.

TOK

Theories are developed after the accumulation of a great deal of data via observation and/or experimentation. Sometimes theories will be abandoned completely because of conflicting evidence.

As this chapter develops and more information about the basic characteristics of cells is learned, some recent findings will be discussed.

Functions of life

All organisms exist in either a unicellular or a multicellular form. Interestingly, all organisms, whether unicellular or multicellular, carry out all the functions of life. These functions include:

- metabolism
- growth
- reproduction
- response
- homeostasis
- nutrition
- excretion.

All of these functions act together to produce a viable living unit. Metabolism includes all the chemical reactions that occur within an organism. Cells have the ability to convert energy from one form into another. Growth may be limited but is always evident in one way or another. Reproduction involves hereditary molecules that can be passed to offspring. Responses to stimuli in the environment are imperative for the survival of an organism. These responses allow an organism to adapt to its environment. Homeostasis refers to the maintenance of a constant internal environment. For example, an organism may have to control fluctuating temperature and acid–base levels to create a constant internal environment. Providing a source of compounds with many chemical bonds that can then be broken down to provide an organism with the energy necessary to maintain life is the basis of nutrition. Excretion is essential to life because it enables those chemical compounds that an organism cannot use or that may be toxic or harmful to it to be released from the organism's system.

Two organisms can be used to demonstrate the functions of life: *Paramecium* and *Chlorella*.

Paramecium is a unicellular member of the kingdom known as the Protista. Study the diagram of a *Paramecium* to become familiar with this organism's basic structure.

Viruses, prions, and viroids are not considered to be living organisms. They cannot carry out the functions of life on their own. However, they may use cells to perpetuate themselves.

The functions of life manifest in different ways in different types of organisms. However, all organisms maintain the same general functions that allow them to continue life. You may see different terms for these functions in other sources.

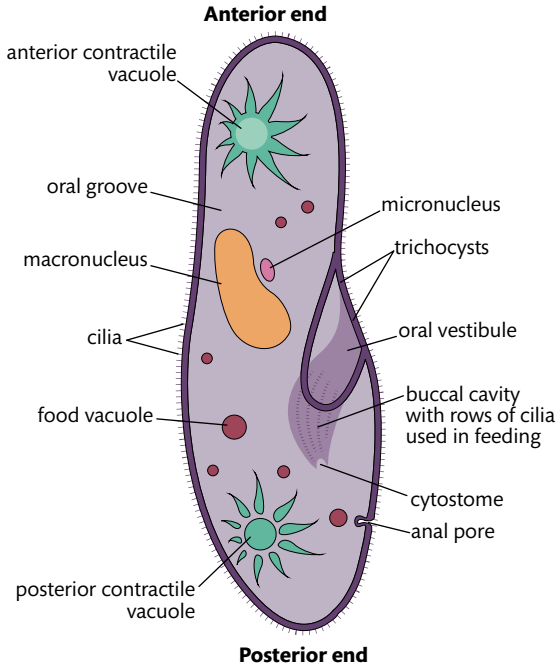


Figure 1.1 *Paramecium*. This single-celled organism may be used to demonstrate several of the functions of life.

CHALLENGE YOURSELF

Answer the following questions about the observations you made in the labs.

1. With the paramecia, the microorganisms should have clustered around the negative pole. Which of the processes of life is demonstrated by this action?
2. You should have seen that when food was added to a culture of paramecia they clustered around the food particles. Which of the functions of life does this represent?
3. After these organisms had used the food particles, what life function would they carry out to get rid of potentially toxic wastes?
4. Two of the structures shown in the diagram of a *Paramecium* (Figure 1.1) are involved in excretion or internal water concentration regulation. They are the anal pore and the contractile vacuole. Conduct some research into the role each of these structures plays in excretion.

***Paramecium* and the functions of life**

Safety alerts: Be cautious of sharp objects. Only use a 9-volt battery as an electrical source. Make sure your instructor checks your set-up before you begin. Wash your hands thoroughly with soap and water before and after the procedure.

Paramecium can be used to demonstrate the functions of life in several ways.

1. Place a number of paramecia into a Syracuse dish or an evaporating dish with positive and negative electrodes of low-voltage electrical charge on opposite sides. A simple 9-volt battery will usually trigger a response. Do not use electricity of a higher voltage, otherwise the organism will be harmed. Low-voltage electricity can be applied for several minutes. The dish should be placed on the stage of a dissecting microscope. A strong magnifying lens may also be used. Describe the movement and final location of the largest population of paramecia.
2. Once this activity has ended, remove the electrodes and add several small, but visible, pieces of hard-boiled egg yolk. Again, using the magnifying instrument make observations of the movement and final location of the paramecia.
3. Finally, to a culture of paramecia add a drop of very dilute acetic acid (vinegar). Once again, report on the movement and final location of the paramecia.
4. When you have finished these tests, your teacher will explain what should be done with the organisms. Respect for life is very important in our studies. The IB policy on animal experimentation must be followed at all times.
5. Using what you know about the functions of life, explain why the paramecia moved in the ways you observed.

The next organism we will look at is *Chlorella*. Compared with *Paramecium*, *Chlorella* has a completely different approach to nutrition. *Chlorella* is a single-celled organism that has one very large structure called a chloroplast inside a cell wall. This structure enables the conversion of the energy in sunlight to a chemical energy form called carbohydrate. This carbohydrate provides the major nutritional source for the organism. Study the diagram of a *Chlorella*.

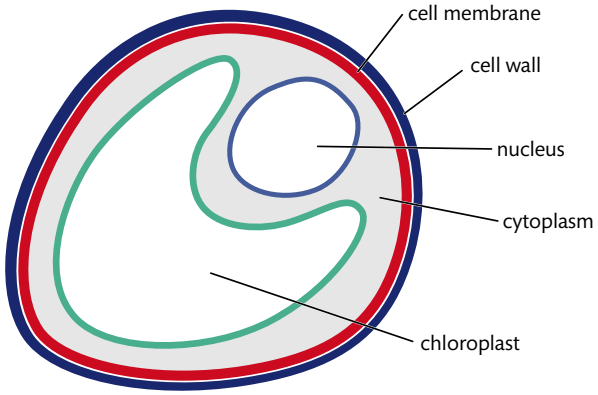


Figure 1.2 *Chlorella*. A common freshwater organism. This organism has been used by many researchers to determine the details of, and the factors that affect, a process known as photosynthesis. The structure labelled chloroplast is especially important in this process.

***Chlorella* and the functions of life**

Safety alerts: Be cautious of sharp objects. Follow classroom rules for use of microscope. Wash your hands thoroughly with soap and water before and after the procedure.

Many classroom practical activities can be carried out with cultures of *Chlorella*. Carry out the following activity.

1. Obtain two depression microscope slides, and place the same number of *Chlorella* organisms in a proper culture medium in each well.
2. Seal a cover slip on each slide with a ring of petroleum jelly.
3. To reduce evaporation further, place each slide in a Petri dish.
4. Place one Petri dish with its slide in sunlight.
5. Place the other Petri dish in complete darkness.
6. Using a microscope, check the numbers of *Chlorella* on each slide for 3 days.
7. Use the functions of life to explain the results observed.
8. An advanced activity can be carried out. Using a culture of *Chlorella*, design an experiment that would allow you to see what colour (wavelength) of light this organism prefers.

Correlation and cause are extremely important in scientific research. A correlation means there is a statistical link relating one variable or factor with another. In the case of a causal relationship, one factor causes another; there must be a scientific process or mechanism connecting the factors with one another.

NATURE OF SCIENCE

Perhaps in the design of the *Chlorella* activity you had an idea based on your previous experiences in science about what the outcome of your procedure would be. This idea is referred to as a hypothesis. Scientists form hypotheses that can be tested by observation and/or experimentation. These tested hypotheses may ultimately serve to simplify and unify existing scientific ideas.

Controlled experiments are the best way to investigate the relationship between two factors or variables. However, this type of experiment is not always possible. In this case, statistical analysis of the data may indicate a correlation. As time and research proceeds, a causal relationship may be seen. Objective data, both qualitative and quantitative, are used to establish relationships whenever possible. It is essential that repeated measurements are taken and that large numbers of readings are taken so that the data collection is reliable. Scientists spend a lot of time working with people from other disciplines in order to gain a greater understanding of their findings. They also read current scientific articles throughout their career in order to gain further insight into their research. Eventually, a researcher may decide to publish his or her findings in an appropriate scientific journal. For this to happen, an article undergoes a peer-review process, which means several scientists working in the same field read the article before it is published to make sure the methodologies and findings are sound and honest.

Cells and sizes

Cells are made up of a number of different subunits. These subunits are often of a particular size, but all are microscopically small. In most cases the use of microscopes

with a high magnification and resolution are needed to observe cells and especially their subunits. Resolution refers to the clarity of a viewed object.

Light microscopes use light, passing through living or dead specimens, to form an image. Stains may be used to make it easier to see any details. Electron microscopes use electrons passing through a dead specimen to form an image and provide us with the greatest magnifications (over 100 000×) and resolution.

Most cells can be up to 100 micrometres (µm) in size. Organelles can be up to 10 µm in size. Bacteria can be up to 1 µm in size. Viruses can be up to 100 nanometres (nm) in size. Cell membranes are 10 nm thick, while molecules are about 1 nm in size. All of these objects are three-dimensional.

Table 1.1 A comparison of light and electron microscopes

Light microscope	Electron microscope
Inexpensive to purchase and operate	Expensive to purchase and operate
Simple and easy specimen preparation	Complex and lengthy specimen preparation
Magnifies up to 2000×	Magnifies over 500 000×
Specimens may be living or dead	Specimens are dead, and must be fixed in a plastic material

Cells and their subunits are so small they are hard to visualize, so it is important to appreciate their relative sizes. Cells are relatively large, and then in decreasing order of size are:

- organelles
- bacteria
- viruses
- membranes
- molecules.

If you want to calculate the actual size of a specimen seen with a microscope, you need to know the diameter of the microscope's field of vision. This can be calculated with a special micrometre, or on a light microscope with a simple ruler. The size of the specimen can then be worked out. Drawings or photographs of specimens are often enlarged. To calculate the magnification of a drawing or photograph, a simple formula is used:

magnification = size of image/by size of specimen.

Scale bars are often used with a micrograph or drawing so that the actual size can be determined. Scale bars and magnification will be addressed in more detail in a later practical activity.

Worked example

The length of an image you are looking at is 50 mm. If the actual length of the subject of the image is 5 µm, what is the magnification of the image you are looking at?

Solution

magnification = 50 mm/5 µm = 50 000 µm/5 µm = 10 000×

Or: magnification = 50 mm/5 µm = 50×10^{-3} m divided by 1×10^{-6} m = 10 000×

Limiting cell size

So, the cell is a small object. You may wonder why cells do not grow to larger sizes, especially as growth is one of the functions of life. There is a principle called the surface area to volume ratio that effectively limits the size of cells. In a cell, the rate of heat and waste production, and rate of resource consumption, are functions of (depend on) its volume. Most of the chemical reactions of life occur inside a cell, and the size of the cell affects the rate of those reactions. The surface of the cell, the membrane, controls what materials move in and out of the cell. A cell with more surface area per unit volume is able to move more materials in and out of the cell, for each unit volume of the cell.

As the width of an object such as a cell increases, the surface area also increases, but at a much slower rate than the volume. This is shown in the following table: the volume increases by a factor calculated by cubing the radius; at the same time, the surface area increases by a factor calculated by squaring the radius.

Table 1.2 Surface area to volume ratios

Factor	Measurement		
Cell radius (*r*)	0.25	0.50	1.25
Surface area	0.79	3.14	19.63
Volume	0.07	0.52	8.18
Surface area : volume ratio	11.29 : 1	6.04 : 1	2.40 : 1

Sphere formulas:

Surface area = (four)(pi)(radius squared)

Volume = (four-thirds)(pi)(radius cubed)

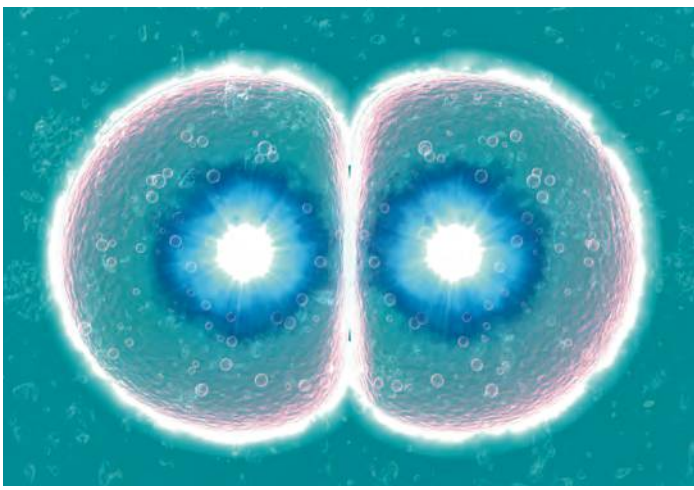
This means that a large cell, compared with a small cell, has relatively less surface area to bring in materials that are needed and to get rid of waste. Because of this, cells are limited in the size they can reach and still be able to carry out the functions of life. Thus large animals do not have larger cells; instead they have more cells.

Cells that are larger in size have modifications that allow them to function efficiently. This is accomplished with changes in shape, such as being long and thin rather than spherical. Some larger cells also have infoldings or outfoldings to increase their surface area relative to their volume.

Cell reproduction and differentiation

One of the functions that many cells have is the ability to reproduce themselves. In multicellular organisms this allows growth to happen. It also means damaged or dead cells can be replaced.

Multicellular organisms usually start their existence as a single cell after some type of sexual reproduction. This single cell has the ability to reproduce at a very rapid rate, and the resulting cells then go through a differentiation process to produce all the required cell types that are necessary for the well-being of the organism. The number of different cell types that can arise from the one original cell can be staggering. This differentiation process is the result of the expression of certain specific genes but not



This is a computer artwork of an egg cell fertilized during *in vitro* fertilization and now undergoing the first cell division.

others. Genes, segments of DNA on a chromosome, enable the production of all the different cells in an organism. Therefore, each cell contains all the genetic information needed for the production of the complete organism. However, each cell will become a specific type of cell depending on which DNA segment becomes active.

Some cells have a greatly reduced ability to reproduce once they become specialized, or lose the ability altogether. Nerve and muscle cells are good examples of this type of cell. Other cells, including epithelial cells such as skin, retain the ability to reproduce rapidly throughout their life. The offspring of these rapidly reproducing cells will then differentiate into the same cell type as the parent.

Cancer cells are examples of cells that undergo extremely rapid reproduction with very little or improper differentiation. The result is a mass of cells (a tumour) with no useful function to the organism.

One of the results of cell reproduction and the subsequent differentiation process that occurs in multicellular organisms is emergent properties. These properties depend on the interactions between all the different parts of a particular biological unit, such as the cell. When you look at the function(s) of each part of a cell, it is less than the overall function of the complete cell. In other words, the whole is more than the sum of its parts. To continue with this emergent concept, a whole multicellular organism is capable of carrying out more functions than the sum of the function(s) each cell is specialized in. The ultimate example of emergence is a collection of inert (non-living) molecules that is capable, when functioning together, of creating a living entity that demonstrates the functions of life.

When discussing the overall functions of a cell, you should focus on the distinctions between living and non-living factors in the environment. It is very useful and productive to refer to the functions of life in such a discussion.

Stem cells

There are populations of cells within organisms that retain their ability to divide and differentiate into various cell types. These cells are called stem cells.

Plants contain such cells in regions of meristematic tissue. Meristematic tissues occur near root and stem tips and are composed of rapidly reproducing cells that produce new cells capable of becoming various types of tissue within that root or stem. Gardeners take advantage of these cells when they take cuttings from stems or roots and use them to propagate new plants.

In the early 1980s, scientists found pluripotent or embryonic stem cells in mice. These stem cells retain the ability to form any type of cell in an organism and can even form a complete organism.

When stem cells divide to form a specific type of tissue, they also produce some daughter cells that stay as stem cells. This enables the continual production of a particular type of tissue. Medical scientists saw the possibilities of using such cells to treat certain human diseases. However, one problem discovered early on in stem cell research was that stem cells cannot be distinguished by their appearance. They can only be isolated from other cells on the basis of their behaviour.

Stem cell research and treatments

Recently some very promising research has been directed towards growing large numbers of embryonic stem cells in culture so that they can be used to replace differentiated cells lost as a result of injury and disease. This involves therapeutic cloning. Parkinson's and Alzheimer's diseases are caused by the loss of proper functioning brain cells, and it is hoped that implanted stem cells could replace many of these lost or defective brain cells, thus relieving the symptoms of the disease. With some forms of diabetes, the pancreas is depleted of essential cells and it is hoped that

a stem cell implant in this organ could have positive effects. As at present most of the research on stem cells is being carried out using mice, it will probably be some time before this approach to treatment becomes widespread in humans.

Stem cells are being utilized in a number of ways by scientists around the world. One area of research involves using human embryonic stem cells in order to understand human development better. This research involves studies of cell division and differentiation. Other scientists are using stem cells to test the safety and effects of new drugs. Information in this area is essential to the understanding of how these drugs might affect differentiating cells in existing organisms. Another very interesting area of study involves cell-based therapies, especially as they may have a positive influence on the treatment of diseases and traumas such as Alzheimer's disease, spinal cord injuries, heart disease, diabetes, burns, and strokes.

In 2005, stem cells were used successfully to help restore the lost insulation of nerve cells in rats, thus resulting in greater mobility in these animals.

However, there is a type of stem cell treatment that has been used successfully in humans for many years. As well as pluripotent stem cells, there are tissue-specific stem cells. These stem cells reside in certain tissue types and can only produce new cells of that particular tissue. For example, blood stem cells have been introduced routinely into humans to replace the damaged bone marrow of some leukaemia patients.

Stargardt's disease is an example of a human condition that is in the early stages of being treated with stem cells. Stargardt's disease is an inherited disease caused by both parents passing on a gene to their offspring that codes for a defect in the processing of vitamin A. Vitamin A is essential for the light-sensitive cells in the retina to function properly. With Stargardt's disease, within the first 20 years of a patient's life he or she begins to lose his or her central vision. Later on, peripheral vision loss occurs, which eventually leads to blindness.

In March 2010, a stem cell treatment was begun that was designed to protect and regenerate photoreceptors in the retina that are damaged by Stargardt's disease. Currently the particular stem cells being used for this treatment in humans are human embryonic stem cells. The study is ongoing, but the early results are promising.

There has been much sharing of data involving stem cell research. However, many nations have banned or restricted research in this area because of local cultural and religious traditions.

There are ethical issues involved in stem cell research. The use of pluripotent stem cells is particularly controversial. These cells are obtained from embryos, largely from laboratories carrying out *in vitro* fertilization (IVF). Harvesting these cells involves the death of an embryo, and some people argue that this is taking a human life. Others argue that this research could result in a significant reduction in human suffering, and is, therefore, totally acceptable.

TOK

How the scientific community conveys information concerning its research to the wider society is very important. The information must be accurate, complete, and understandable, so that society can make informed decisions regarding the appropriateness of the research. There is a need to balance the very great opportunities of this type of research with the potential risks. Recently, there has been evidence that some types of cancer may be caused by stem cells undergoing a cancer-like or malignant transformation. Where do you stand in the debate about the nature of stem cell research? How do you feel about the source of pluripotent stem cells?

Exercises

1. How is the excretion of metabolic wastes from cells related to the concept of the surface area to volume ratio?
2. Explain how the function of life known as nutrition differs in *Paramecium* compared with the green alga *Chlorella*.
3. How does specialization in muscle and nerve cells affect their ability to reproduce?
4. What would prevent stem cells from other species being successful in humans?

NATURE OF SCIENCE

Developments in scientific research follow improvements in apparatus: the invention of electron microscopes led to greater understanding of cell structure.

1.2 The ultrastructure of cells

Understandings:

- Prokaryotes have a simple cell structure without compartmentalization.
- Eukaryotes have a compartmentalized cell structure.
- Electron microscopes have a much higher magnification than light microscopes.

Applications and skills:

- Application: Structure and function of organelles within exocrine gland cells of the pancreas and within palisade mesophyll cells of the leaf.
- Application: Prokaryotes divide by binary fission.
- Skill: Drawing of the ultrastructure of prokaryotic cells based on electron micrographs.
- Skill: Drawing of the ultrastructure of eukaryotic cells based on electron micrographs.
- Skill: Interpretation of electron micrographs to identify organelles and deduce the function of specialized cells.

Guidance

- *Drawings of prokaryotic cells should show the cell wall, pili, and flagella, and plasma membrane enclosing cytoplasm that contains 70S ribosomes and a nucleoid with naked DNA.*
- *Drawings of eukaryotic cells should show a plasma membrane enclosing cytoplasm that contains 80S ribosomes and a nucleus, mitochondria and other membrane-bound organelles are present in the cytoplasm. Some eukaryotic cells have a cell wall.*

What is a prokaryotic cell?

Becoming familiar with common prefixes, suffixes and word roots will help you understand biological terms. For example, the word prokaryotic comes from the Greek word 'pro', which means 'before', and 'karyon', which means kernel, referring to the nucleus.

After extensive studies of cells, it has become apparent that all cells use some common molecular mechanisms. There are huge differences between different forms of life but cells are the basic unit and different cells have many characteristics in common. Cells are often divided into particular groups based on major characteristics. One such division separates cells into two groups: prokaryotic and eukaryotic cells. Prokaryotic cells are much smaller and simpler than eukaryotic cells. In fact, most prokaryotic cells are less than 1 μm in diameter. Because of this, and many other reasons that will be discussed later, the prokaryotic cells are thought to have appeared on Earth first. As bacteria are prokaryotic cells, you can see that such cells play a large role in the world today.

Features of prokaryotic cells

Bacteria and members of a group referred to as Archaea are made up of prokaryotic cells and are called prokaryotes. The vast majority of these organisms do not cause disease and are not pathogenic (disease-causing).

Study the figure of a prokaryotic cell (Figure 1.3) and make sure you can identify:

- the cell wall
- the plasma membrane
- flagella
- pili
- ribosomes
- the nucleoid (a region containing free DNA).

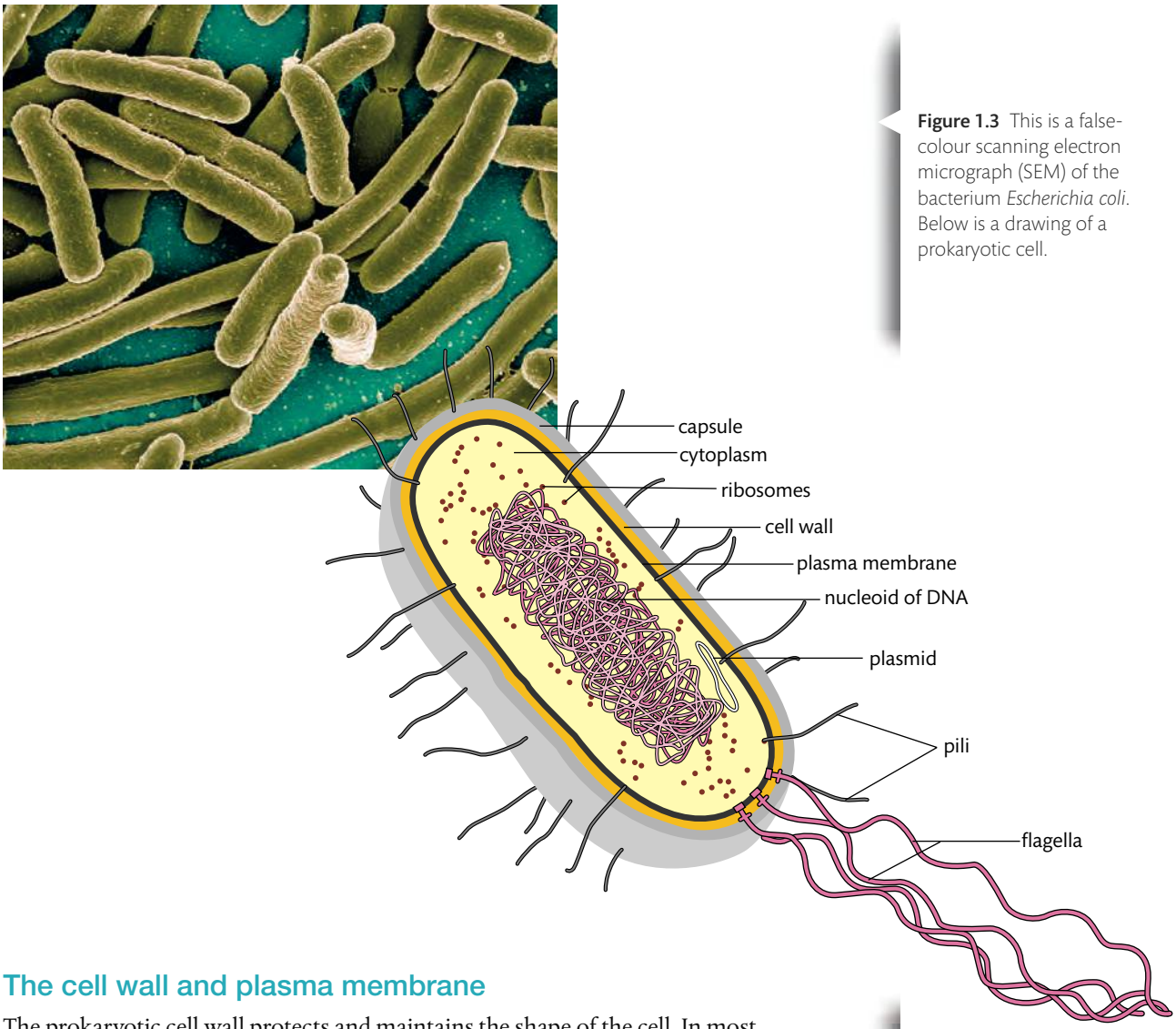


Figure 1.3 This is a false-colour scanning electron micrograph (SEM) of the bacterium *Escherichia coli*. Below is a drawing of a prokaryotic cell.

The cell wall and plasma membrane

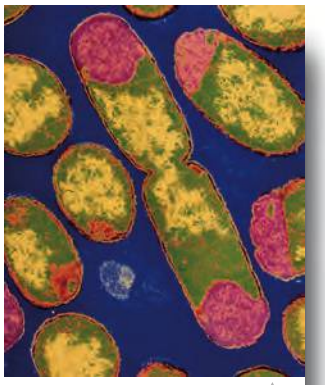
The prokaryotic cell wall protects and maintains the shape of the cell. In most prokaryotic cells this wall is composed of a carbohydrate–protein complex called peptidoglycan. Some bacteria have an additional layer of a type of polysaccharide outside the cell wall. This layer makes it possible for some bacteria to adhere to structures such as teeth, skin and food.

The plasma membrane is found just inside the cell wall and is similar in composition to the membranes of eukaryotic cells. To a large extent the plasma membrane controls the movement of materials into and out of the cell, and it plays a role in binary fission of the prokaryotic cell. The cytoplasm occupies the complete interior of the cell. The most visible structure with a microscope capable of high magnification is the chromosome or a molecule of DNA. There is no compartmentalization within the cytoplasm because there are no internal membranes other than the plasma membrane. Therefore, all cellular processes within prokaryotic cells occur within the cytoplasm.

If there is no compartmentalization within prokaryotic cells, chemical reactions are not isolated from one another. This may limit the cell's development and efficiency because of possible interference between the reactions.

Pili and flagella

Some bacterial cells contain hair-like growths on the outside of the cell wall. These structures are called pili and can be used for attachment. However, their main function



This is a false-colour transmission electron micrograph (TEM) showing *Escherichia coli* dividing by binary fission.

is joining bacterial cells in preparation for the transfer of DNA from one cell to another (sexual reproduction).

Some bacteria have flagella (plural) or a flagellum (singular), which are longer than pili. Flagella allow a cell to move.

Ribosomes

Ribosomes occur in all prokaryotic cells and they function as sites of protein synthesis. These small structures occur in very large numbers in cells that produce a lot of protein, and, when numerous, they give a granular appearance to an electron micrograph of a prokaryotic cell.

The nucleoid region

The nucleoid region of a bacterial cell is non-compartmentalized and contains a single, long, continuous, circular thread of DNA, the bacterial chromosome. Therefore this region is involved with cell control and reproduction. In addition to the bacterial chromosome, bacteria may also contain plasmids. These small, circular, DNA molecules are not connected to the main bacterial chromosome. The plasmids replicate independently of the chromosomal DNA. Plasmid DNA is not required by the cell under normal conditions but it may help the cell adapt to unusual circumstances.

The importance of plasmids in prokaryotic cells will be discussed fully in Chapter 3. Plasmids have very important roles to play in some techniques involving genetic engineering/modification.

Binary fission

Prokaryotic cells divide by a very simple process called binary fission. During this process, the DNA is copied, the two daughter chromosomes become attached to different regions on the plasma membrane, and the cell divides into two genetically identical daughter cells. This divisional process includes an elongation of the cell and a partitioning of the newly produced DNA by microtubule-like fibres called FtsZ.

Some types of bacteria go through binary fission every 20 minutes when conditions are ideal. This results in huge populations and greater potential for infections. Refrigeration of foods is often used to reduce ideal conditions for bacteria. This results in lower bacterial counts in our food and less chance of infection/food poisoning.

Very often in IB, laboratory tests and examinations will require you to draw an object or organism. Follow the guidelines given below when completing any drawing.

- The size should be appropriate for the complexity of the drawing.
- Correct positioning of structures is essential.
- The outline of structures should be continuous unless gaps or pores are present in the actual border or structure.
- Proportions are important.
- The relative numbers of parts are important.
- Draw in pencil first so that mistakes can be corrected. Write on or label the final drawing in black ink.
- Labelling must be included on all drawings unless the question tells you not to.
- Lines from labels to parts on a drawing should be straight and should never cross.
- In IB exams, boxes are provided for drawings. Do not draw or write outside the box as this area will not be scanned or marked.

CHALLENGE YOURSELF

5 Prepare a drawing of the ultrastructure of a prokaryotic cell based on electron micrographs. Make sure you follow the guidelines given for drawings.

Summary

Here is a list of the major distinguishing characteristics of prokaryotic cells.

- Their DNA is not enclosed within a membrane and forms one circular chromosome.
- Their DNA is free; it is not attached to proteins.
- They lack membrane-bound organelles. Ribosomes are complex structures within the plasma membrane, but they have no exterior membrane.

- Their cell wall is made up of a compound called peptidoglycan.
- They usually divide by binary fission, a simple form of cell division.
- They are characteristically small in size, usually between 1 and 10 µm.

What is a eukaryotic cell?

Whereas prokaryotic cells occur in bacteria, eukaryotic cells occur in organisms such as algae, protozoa, fungi, plants, and animals. Examine the following diagrams and pictures.

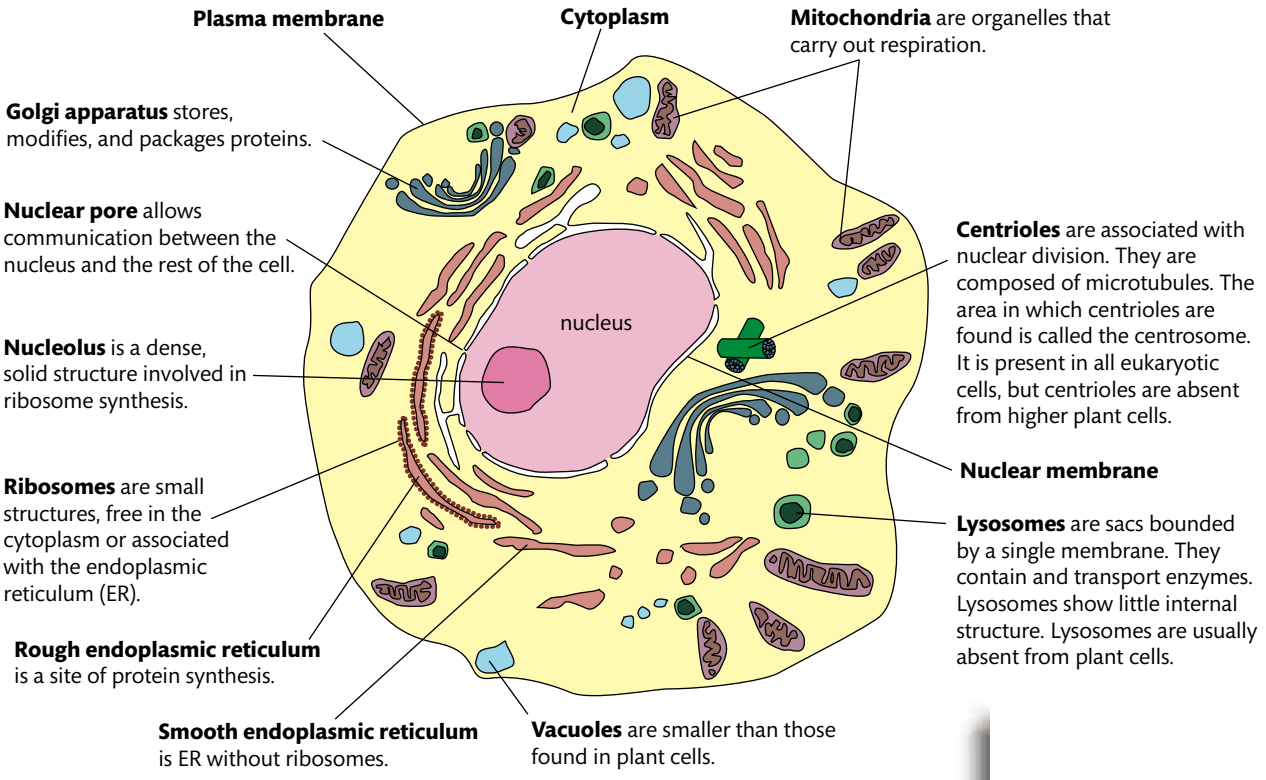
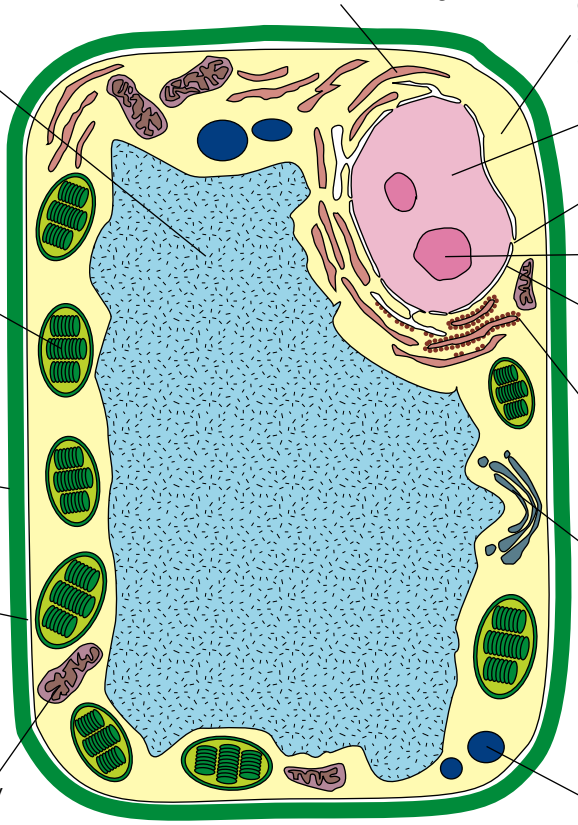


Figure 1.4 Look at this drawing of a typical animal cell and compare it with Figure 1.5.

Eukaryotic cells range in diameter from 5 to 100 µm. A 'kernel' or nucleus is usually noticeable in the cytoplasm. Other organelles may be visible within the cell if you have a microscope with a high enough magnification and resolution. Organelles are non-cellular structures that carry out specific functions (a bit like organs in multicellular organisms); different types of cell often have different organelles. These structures enable compartmentalization in eukaryotic cells, which is not a characteristic of prokaryotic cells. Compartmentalization enables different chemical reactions to be separated, which is especially important when adjacent chemical reactions are incompatible. Compartmentalization also allows chemicals for specific reactions to be isolated; this isolation results in increased efficiency.

The term 'eukaryote' comes from the Greek word 'eukaryon' meaning true kernel or true nucleus.



Endoplasmic reticulum (ER) is a network of tubes and flattened sacs. ER connects with the plasma membrane and the nuclear membrane and may be smooth or have attached ribosomes (rough ER).

Central vacuole has storage and hydrolytic functions

Cytoplasm contains dissolved substances, enzymes, and the cell organelles.

Nucleus contains most of the cell's DNA.

Nuclear pore

Nucleolus

Chloroplasts are specialized plastids containing the green pigment chlorophyll. They consist of grana within the colourless stroma. They are the sites for photosynthesis.

Nuclear membrane is a double-layered structure.

Ribosomes are small (20 nm) structures that manufacture proteins. They may be free in the cytoplasm or associated with the surface of the endoplasmic reticulum.

Cell wall is a semi-rigid structure composed mainly of cellulose.

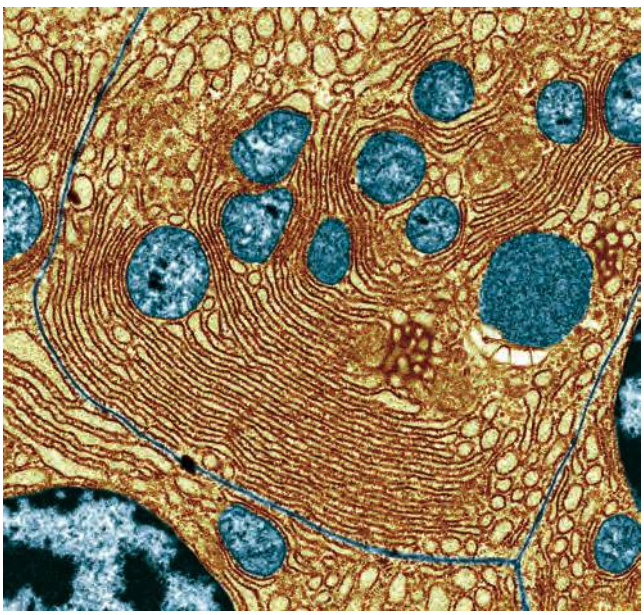
Golgi apparatus

Plasma membrane is inside the cell wall.

Mitochondria are bounded by a double membrane. They are energy transformers.

Starch granules are composed of carbohydrate stored in amyloplasts.

Figure 1.5 What is different and what is similar between this typical plant cell and Figure 1.4?



A TEM of a pancreatic exocrine cell. Can you tell this is an animal cell? Locate as many of the structures of an animal cell as you can. How do the structures of this cell reflect the overall functions of the pancreas?

A TEM of a cell from the mesophyll region of a leaf. How do you know this is a plant cell? Locate as many of the structures of a plant cell as you can. What cell structures reflect most the unique abilities of a plant cell?

As you read about the organelles of eukaryotic cells below, refer back to the figures above and on page 16 and add more names of organelles. Also, be certain to note which organelles are common to both types of cells and which organelles occur in only one of the two types.

Organelles of eukaryotic cells

Common organelles include the following (see Figures 1.4 and 1.5):

- endoplasmic reticulum
- ribosomes
- lysosomes (not usually found in plant cells)
- Golgi apparatus
- mitochondria
- nucleus
- chloroplasts (only in plant and algal cells)
- centrosomes (in all eukaryotic cells, but centrioles are not found in some plant cells)
- vacuoles.

The microscope has given us an insight into the structure and function of the following eukaryotic cell organelles and characteristics.

Cytoplasm

All eukaryotic cells have a region called the cytoplasm that occurs inside the plasma membrane or the outer boundary of the cell. It is in this region that the organelles are found. The fluid portion of the cytoplasm around the organelles is called the cytosol.

Endoplasmic reticulum

The endoplasmic reticulum (ER) is an extensive network of tubules or channels that extends most everywhere in the cell, from the nucleus to the plasma membrane. Its structure enables its function, which is the transportation of materials throughout the internal region of the cell. There are two general types of ER: smooth ER and rough ER. Smooth ER does not have any of the organelles called ribosomes on its exterior surface. Rough ER has ribosomes on its exterior.

Microscopes have a rich history of international development. Glass lenses were used in the first century by the Romans to magnify objects. Salvino D'Armate, an Italian, made an eye glass in the 13th century that allowed magnification for one eye. Two Dutch eyeglass makers, Hans Jansen and his son Zacharias Jansen, in the 1590s, produced the first compound microscope by putting two lenses together. Anton van Leeuwenhoek, also Dutch, greatly improved the Jansen compound microscope in the 1600s. Since this beginning, many individuals in many different countries of the world have worked to make the present-day microscope extremely effective in the study of the cell and other small structures. Modern technology allowing extensive communication has been extremely important in the continual improvement of the present-day microscope.

TOK

Visual illusions are the result of sensory-derived images that differ from objective reality. M. C. Escher's *Waterfall* is a prime example of an optical illusion. In his print water seems to flow downhill on its way to the 'top' of the waterfall. How can the repeating of experiments in science decrease the chances of an illusion? Do you feel modern technology is decreasing the chances of these visual illusions occurring in modern-day science research?

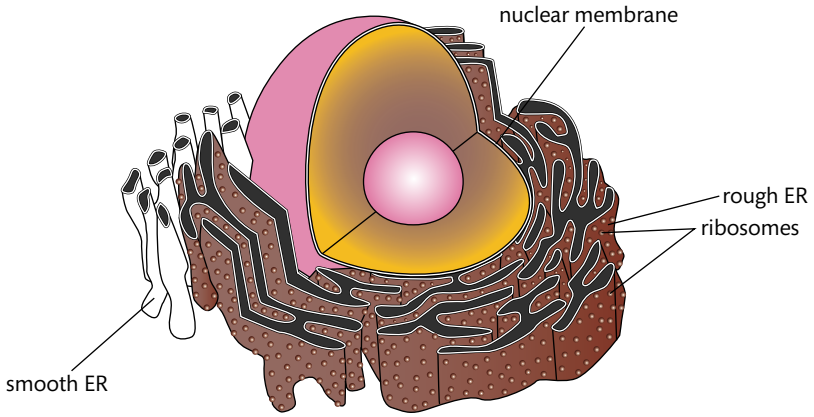


Figure 1.6 Smooth ER and rough ER.

Smooth ER has many unique enzymes embedded on its surface. Its functions are:

- the production of membrane phospholipids and cellular lipids
- the production of sex hormones such as testosterone and oestrogen
- detoxification of drugs in the liver
- the storage of calcium ions in muscle cells, needed for contraction of muscle cells
- transportation of lipid-based compounds
- helping the liver release glucose into the bloodstream when needed.

Rough ER has ribosomes on the exterior of the channels. These ribosomes are involved in protein synthesis. Therefore, this type of ER is involved in protein development and transport. These proteins may become parts of membranes, enzymes, or even messengers between cells. Most cells contain both types of ER, with the rough ER being closer to the nuclear membrane.

The letter S used in the measurement of ribosomes refers to Svedberg units, which indicate the relative rate of sedimentation during high-speed centrifugation. The higher the S value, the quicker the structure will become part of the sediment and the more mass it will have.

Ribosomes

Ribosomes are unique structures that do not have an exterior membrane. They carry out protein synthesis within the cell. These structures may be found free in the cytoplasm or they may be attached to the surface of ER. They are always composed of a type of RNA and protein. You will recall that prokaryotic cells also contain ribosomes. However, the ribosomes of eukaryotic cells are larger and denser that those of prokaryotic cells. Ribosomes are composed of two subunits. These subunits together equal 80S. The ribosomes in prokaryotic cells are also composed of two subunits, but they only equal 70S.

Lysosomes

Lysosomes are intracellular digestive centres that arise from the Golgi apparatus. A lysosome does not have any internal structures. Lysosomes are sacs bounded by a single membrane that contain as many as 40 different enzymes. The enzymes are all hydrolytic and catalyse the breakdown of proteins, nucleic acids, lipids, and carbohydrates. Lysosomes fuse with old or damaged organelles from within the cell to break them down, so that recycling of the components can occur. Lysosomes are also involved in the breakdown of materials that may be brought into a cell by phagocytosis. Phagocytosis is a type of endocytosis that is explained on page 36 in Section 1.4. The interior environment of a functioning lysosome is acidic; this acidic environment is necessary for the enzymes to hydrolyse large molecules.

Golgi apparatus

The Golgi apparatus consists of what appears to be flattened sacs called cisternae, which are stacked one on top of another. This organelle functions in the collection, packaging, modification, and distribution of materials synthesized in the cell. One side of the apparatus is near the rough ER, called the *cis* side. It receives products from the ER. These products then move into the cisternae of the Golgi apparatus. They continue to move to the discharging or opposite side, the *trans* side. Small sacs called vesicles can then be seen coming off the *trans* side. These vesicles carry modified materials to wherever they are needed inside or outside the cell. This organelle is especially prevalent in glandular cells, such as those in the pancreas, which manufacture and secrete substances.

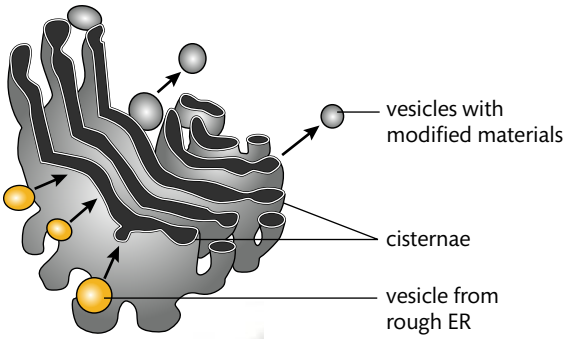


Figure 1.7 In this drawing of the Golgi apparatus, the movement of the vesicles is shown by arrows. Can you identify which side is the *cis* side and which is the *trans* side?

Mitochondria

Mitochondria (singular mitochondrion) are rod-shaped organelles that appear throughout the cytoplasm. They are close in size to a bacterial cell. Mitochondria have their own DNA, a circular chromosome similar to that in bacterial cells, allowing them some independence within a cell. They have a double membrane: the outer membrane is smooth, but the inner membrane is folded into cristae (singular crista). Inside the inner membrane is a semi-fluid substance called the matrix. An area called the inner membrane space lies between the two membranes. The cristae provide a huge surface area within which the chemical reactions characteristic of the mitochondria occur. Most mitochondrial reactions involve the production of usable cellular energy called adenosine triphosphate (ATP). Because of this, the mitochondria are often called the powerhouse of a cell. This organelle also produces and contains its own ribosomes; these ribosomes are of the 70S type. Cells that have high energy requirements, such as muscle cells, have large numbers of mitochondria.

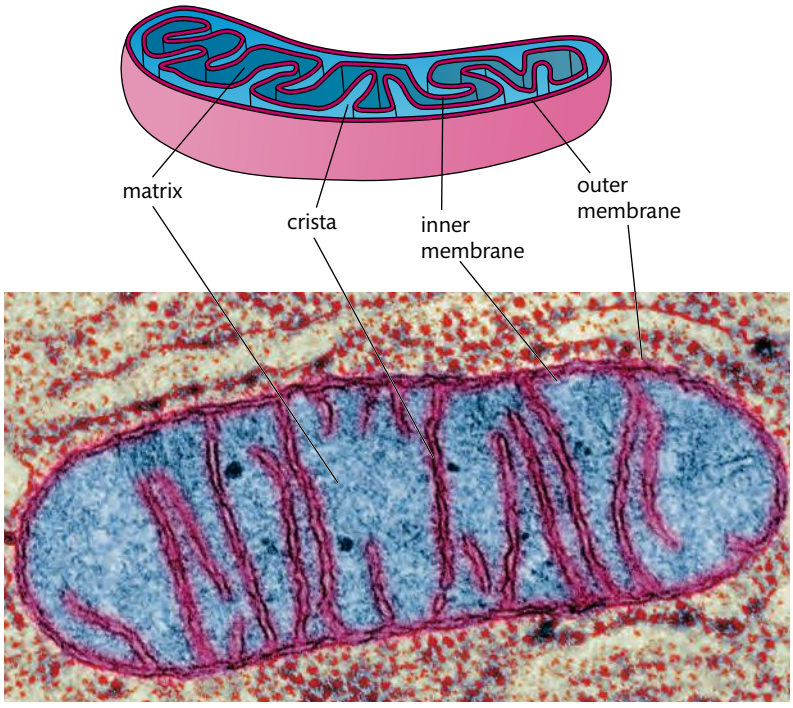


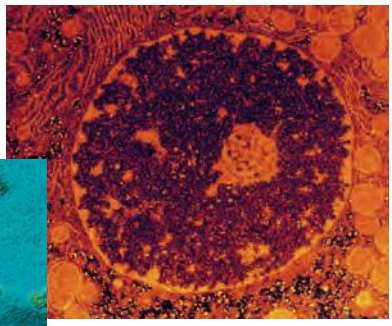
Figure 1.8 Compare this drawing of a mitochondrion with the false-colour TEM of a mitochondrion.

Nucleus

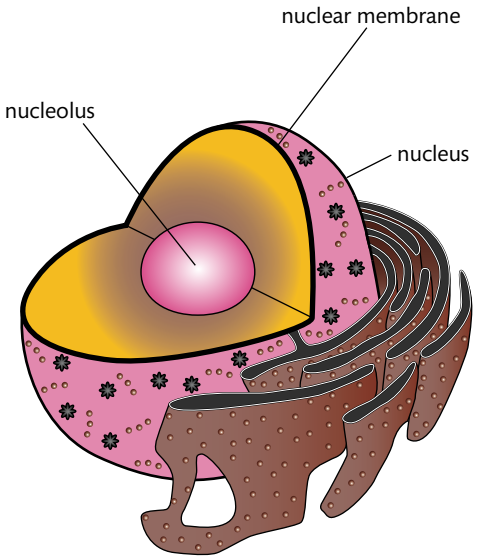
The nucleus in eukaryotic cells is an isolated region where the DNA resides. It is bordered by a double membrane referred to as the nuclear envelope. This membrane allows compartmentalization of the eukaryotic DNA, thus providing an area where DNA can carry out its functions without being affected by processes occurring in other parts of the cell. The nuclear membrane does not provide complete isolation because it has numerous pores that allow communication with the cell's cytoplasm.

Figure 1.9 The nucleus has a double membrane with pores and contains a nucleolus.

False-colour TEM showing pores in the nuclear membrane.

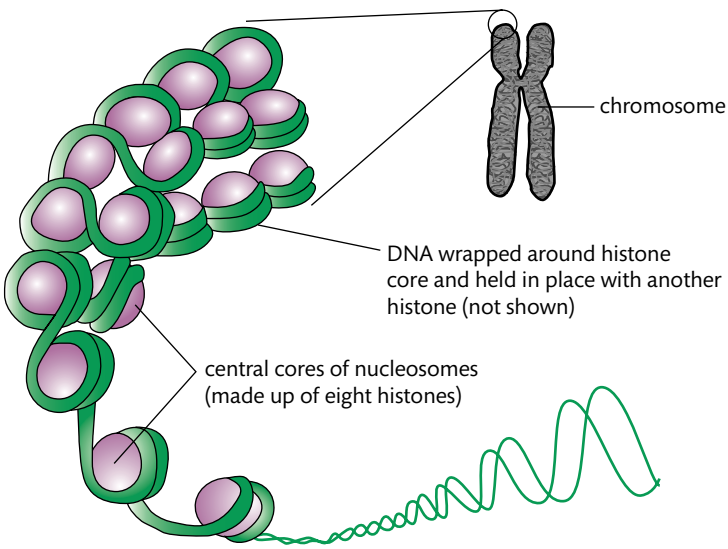


False-colour TEM showing nucleus and nucleolus.



The DNA of a eukaryotic cell often occurs in the form of chromosomes; chromosomes vary in number depending on the species. Chromosomes carry all the information that is necessary for the cell to exist; this allows an organism to survive, whether it is unicellular or multicellular. The DNA is the genetic material of the cell. It enables certain traits to be passed on to the next generation. When the cell is not in the process

Figure 1.10 This drawing shows how DNA is packaged into chromosomes.



of dividing, the chromosomes are not present as visible structures. During this phase the cell's DNA is in the form of chromatin. Chromatin is formed of strands of DNA and proteins called histones. The DNA and histone combination often results in structures called a nucleosome. A nucleosome consists of eight spherical histones with a strand of DNA wrapped around them and secured with a ninth histone. This produces a structure that resembles a string of beads. A chromosome is a highly coiled structure of many nucleosomes.

The nucleus is often located centrally within the cell's cytoplasm, although in some cell types it is pushed to one side or the other. The side position is characteristic of plant cells because these cells often have a large central vacuole. Most eukaryotic cells possess a single nucleus, but some do not have a nucleus at all, and some have multiple nuclei. Without a nucleus, cells cannot reproduce. The loss of reproductive ability is often paired with increased specialization to carry out a certain function. For example, human red blood cells do not have nuclei: they are specialized to transport respiratory gases. Most nuclei also include one or more dark areas called nucleoli (singular nucleolus). Ribosome molecules are manufactured in the nucleolus. The molecules pass through the nuclear envelope before assembling as ribosomes.

Chloroplasts

Chloroplasts occur only in algae and plant cells. The chloroplast contains a double membrane and is about the same size as a bacterial cell. Like the mitochondrion, a chloroplast contains its own DNA and 70S ribosomes. The DNA of a chloroplast takes the form of a ring.

You should note all the characteristics that chloroplasts and mitochondria have in common with prokaryotic cells.

As well as DNA and ribosomes, the interior of a chloroplast includes the grana (singular granum), the thylakoids, and the stroma, which are labelled in Figure 1.11. A granum is made up of numerous thylakoids stacked like a pile of coins. The thylakoids are flattened membrane sacs with components necessary for the absorption of light. Absorption of light is the first step in the process of photosynthesis. The fluid stroma is similar to the cytosol of the cell. It occurs outside the grana but within the double membrane. Stroma contains many enzymes and chemicals that are necessary to complete the process of photosynthesis. Like mitochondria, chloroplasts are capable of reproducing independently of a cell.

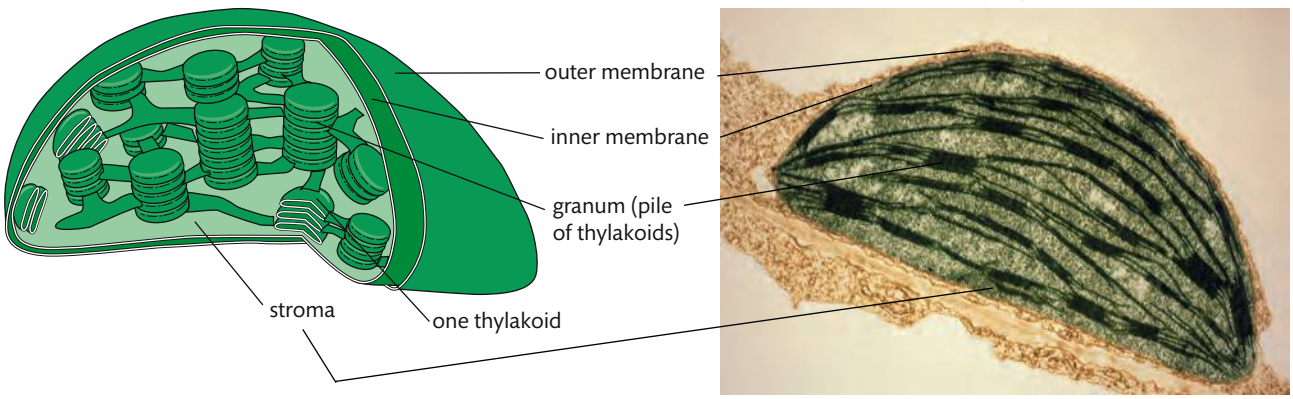
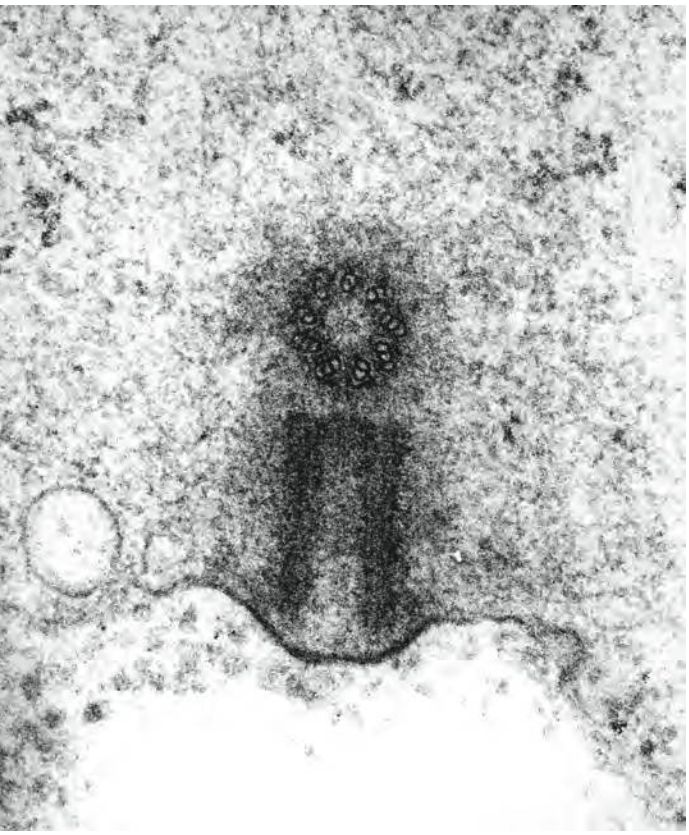


Figure 1.11 Compare the drawing of a chloroplast with this TEM of a chloroplast.



This TEM shows the two centrioles of a centrosome.

Centrosome

The centrosome occurs in all eukaryotic cells. Generally, it consists of a pair of centrioles at right angles to one another. These centrioles are involved with the assembly of microtubules, which are important to a cell because they provide structure and allow movement. Microtubules are also important for cell division. Cells from higher plants, plants that are thought to have evolved later, produce microtubules even though they do not have centrioles. The centrosome is located at one end of the cell close to the nucleus.

Vacuoles

Vacuoles are storage organelles that are usually formed from the Golgi apparatus. They are membrane-bound and have many possible functions. They occupy a very large space inside the cells of most plants. They may store a number of different substances, including potential food (to provide nutrition), metabolic waste and toxins (to be expelled from the cell), and water. Vacuoles enable cells to have higher surface area to volume ratios even at larger sizes. In plants, they allow the uptake of water, which provides rigidity to the organism.

A comparison of prokaryotic and eukaryotic cells

A table is a good way to summarize the differences between prokaryotic and eukaryotic cells.

Table 1.3 Comparing prokaryotic and eukaryotic cells

Prokaryotic cells	Eukaryotic cells
DNA in a ring form without protein	DNA with proteins as chromosomes/chromatin
DNA free in the cytoplasm (nucleoid region)	DNA enclosed within a nuclear envelope (nucleus)
No mitochondria	Mitochondria present
70S ribosomes	80S ribosomes
No internal compartmentalization to form organelles	Internal compartmentalization present to form many types of organelles
Size less than 10 μm	Size more than 10 μm

When comparing items, be certain to state the characteristic of each type of item, as shown in the table for prokaryotic and eukaryotic cells.

If asked to state the similarities between the two types of cells, make sure you include the following:

- both types of cell have some sort of outside boundary that always involves a plasma membrane
- both types of cell carry out all the functions of life
- DNA is present in both cell types.

A comparison of plant and animal cells and their extracellular components

We will now look at how to compare two general types of eukaryotic cell: plant and animal cells. A table like the one below can be used to highlight the differences. However, do not forget to also recognize the similarities between the two cell types.

Table 1.4 Comparing plant and animal cells

Plant cells	Animal cells
The exterior of the cell includes an outer cell wall with a plasma membrane just inside	The exterior of the cell only includes a plasma membrane. There is no cell wall
Chloroplasts are present in the cytoplasm area	There are no chloroplasts
Large centrally located vacuoles are present	Vacuoles are not usually present or are small
Carbohydrates are stored as starch	Carbohydrates are stored as glycogen
Do not contain centrioles within a centrosome area	Contain centrioles within a centrosome area
Because a rigid cell wall is present, this cell type has a fixed, often angular, shape	Without a cell wall, this cell is flexible and more likely to be a rounded shape

Most cell organelles are present in both plant and animal cells. When an organelle is present in both types of cell, it usually has the same structure and function. For example, both cell types contain mitochondria that possess cristae, a matrix, and a double membrane. Also, in both cell types, the mitochondria function in the production of ATP for use by the cell.

The outermost region of various cell types is often unique to that cell type, as shown by the following table.

Table 1.5 Outermost parts of different cells

Cell	Outermost part
Bacteria	Cell wall of peptidoglycan
Fungi	Cell wall of chitin
Yeasts	Cell wall of glucan and mannan
Algae	Cell wall of cellulose
Plants	Cell wall of cellulose
Animals	No cell wall, instead a plasma membrane that secretes a mixture of sugar and proteins called glycoproteins that forms the extracellular matrix

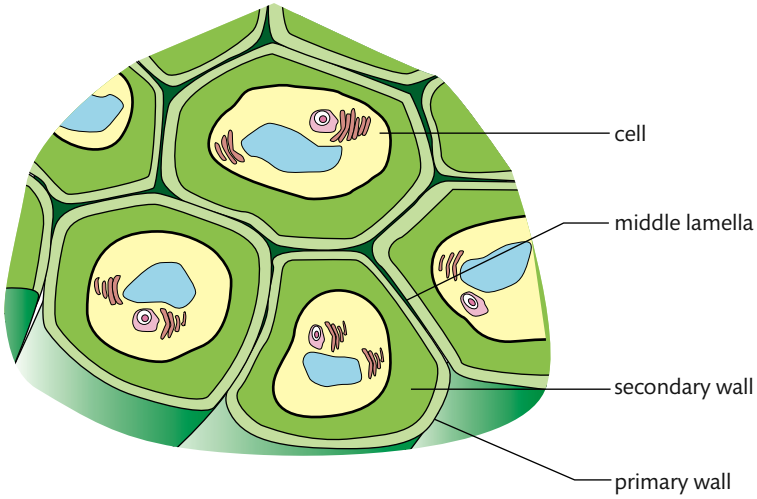
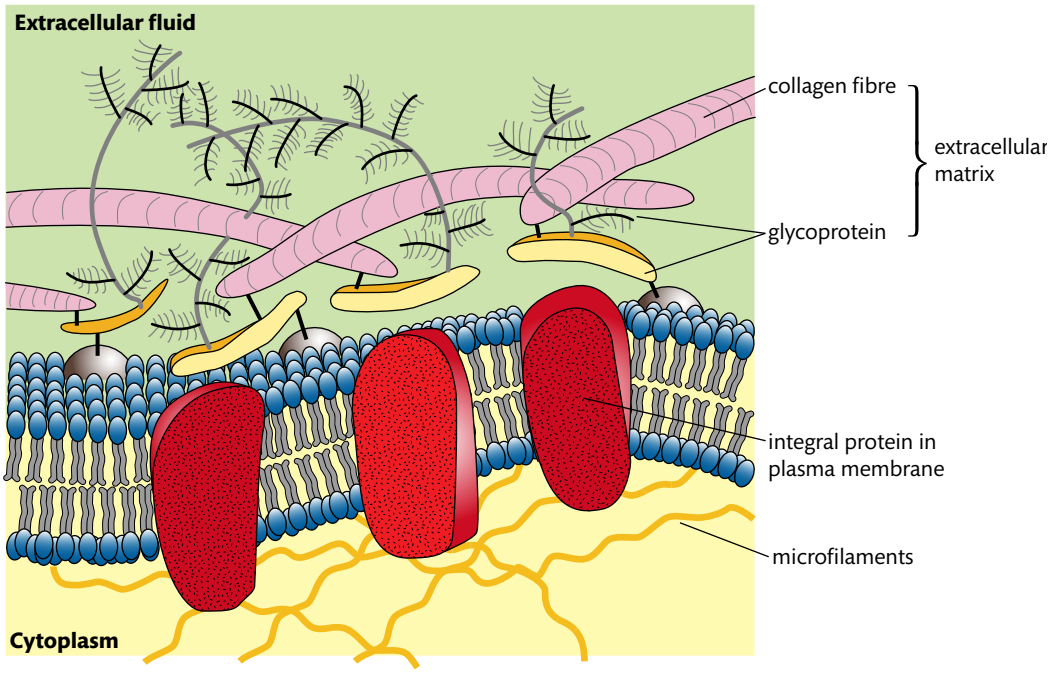


Figure 1.12 This drawing of a section through plant cells shows the primary walls, middle lamella and secondary walls.

Whenever a cell wall is present, it is involved in maintaining cell shape. It also helps regulate water uptake. Because of its rigidity it will only allow a certain amount of water to enter the cell. In plants, when an adequate amount of water is inside the cell, there is pressure against the cell wall. This pressure helps support the plant vertically.

The extracellular matrix (ECM) of many animal cells is composed of collagen fibres plus a combination of sugars and proteins called glycoproteins. These form fibre-like structures that anchor the matrix to the plasma membrane. This strengthens the plasma membrane and allows attachments between adjacent cells. The ECM allows cell-to-cell interactions, possibly altering gene expression and enabling the coordination of cell actions within the tissue. Many researchers think the ECM is involved in directing stem cells to differentiate. Cell migration and movement also appear to be, at least partially, the result of interactions in this area.

Figure 1.13 This is a drawing of the extracellular matrix of an animal cell.



The use of a light microscope to investigate cells and cell structure sizes

Safety alerts: Be very cautious with sharp instruments. Wash your hands thoroughly with soap and water before and after handling cell sources. Follow all additional teacher safety directives.

This practical will develop your skill in using a microscope, allow you to observe some common cells microscopically, and demonstrate ways to calculate the size of cells and cell parts. There are many different types of compound light microscope. Before beginning this practical, it is essential you understand how to use your school's microscopes properly. As well as a microscope, other materials necessary for this practical include microscope slides, cover glasses, methylene blue in a dropper bottle, water in a dropper bottle, a plastic ruler, toothpicks, and several sources of cells.

1 Determine the total magnification of each objective lens

Because you are using a compound microscope, there are two types of lens present. One is the ocular lens and the other is the objective lens. Each of these lenses has a number on it followed by an ×. These numbers represent the magnification of that particular lens. To determine the total magnification of an object being examined with the microscope, multiply the power of the ocular lens by the power of the objective lens. Carry out this procedure for each of the microscope objective lenses you use and record the information required in the following table.

Table 1.6 Microscope total magnification and diameter of field of view

Power of ocular lens	Power of objective lens	Total magnification	Diameter of field of view (mm)	Diameter of field of view (μm)
Low				
Medium				
High				

2 Determine the diameter of the field of view

The field of view (field of vision) is the circular area you can see when you look through the ocular lens of a microscope. It is important to know its diameter. One way to determine this is to place a plastic ruler under the low-power objective lens so that it crosses the diameter of the field of view. Observe and record the diameter in millimetres in the above table. Repeat the same procedure for the next two objectives to determine the diameter of their field of view. Instead of using a ruler for the two higher power objectives, you can use proportions to determine the field of view by comparing their diameters with the diameter determined for the lowest power. Convert millimetre (mm) measurements to microns (micrometres; μm):

$$1 \text{ mm} = 1000 \text{ μm}$$

3 Observing and determining the sizes of cells

(a) You will now look at several types of cells. Prepared slides may be used or you can make your own wet-mount slides. Your teacher will provide the information you need to produce a wet mount.

(b) Some ideas for producing your own wet-mount slides include: the inside epidermal layer from the bulb of an onion; *Elodea* leaf cells; *Anabaena* (an aquatic cyanobacterium); cheek cells from inside your mouth; scraped soft banana tissue.

(c) Whatever cells are used, study them carefully, noting any internal structures, and their size in relation to the rest of the cell and its visible parts. Using a stain such as methylene blue or iodine often means you can see the parts of a cell more clearly. Use any resources available, including texts and the internet, to identify any structures you can see.

(d) For each cell type you observe, complete the following steps.

(i) Using a pencil, draw several typical cells seen in the field of view. Label any visible cell structures.

(ii) Carefully and accurately make a scale drawing of these cells and any visible internal parts.

(iii) Beside each drawing include the:

- total magnification
- diameter of the field of view
- estimated length of an individual cell.

To figure out the length of one cell, divide the diameter of the field of view by the number of cells that cross the diameter of the field of view. This value should be recorded in microns (μm).

Another way to determine the size of objects in the field of view of a microscope is to use an eyepiece graticule. Graticules must be calibrated. To calibrate a graticule, a plastic millimetre ruler or a graduated slide can be used. While using the lowest power objective lens, move the graduated slide until the graticule scale and the graduated slide scale align. The size of the graticule units can now be determined. You can follow the same procedure to calibrate the other two objectives, or you can calculate the other calibrations. Once you have calibrated the graticule, it can be used to take accurate measurements of the object being viewed.

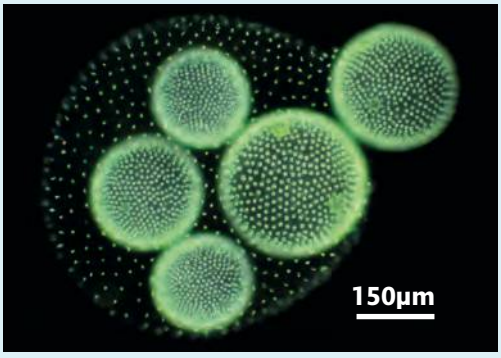
4 Microscope magnification and cell size

We will complete this activity with some problems involving cell size and magnification. Use this general formula for calculating magnification:

magnification = drawing size/actual size

(a) An organism has an actual length of 0.01 mm. If you draw a diagram that is 50 mm, what is the magnification of your drawing?

(b) Scale bars are lines added to a micrograph (the photograph of an image under a microscope) or a drawing to represent the actual size of the structures. For example, a 25-μm bar would represent the size of a 25-μm image. The picture below shows an image of several *Volvox* seen in a microscope field of view. Use the scale bar to determine the approximate size of the three central, fully shown *Volvox*.



(c) An organism has an actual length of 0.05 mm. If you use a scale of 1 : 200, what will the size of your drawing of the organism be?

(d) You should look at more images of micrographs on the internet to develop your skills in determining the sizes of cells and cell structures. Be certain to include electron micrographs in your practice.

Exercises

5 What is a disadvantage to prokaryotic cells of having their DNA free in the cytoplasm without a nuclear membrane?

6 What structures are involved in sexual reproduction in prokaryotic cells?

7 Dental plaque involves the presence of bacteria. Explain how the bacteria are able to attach firmly to teeth such that the bacteria can only be removed with scraping.

8 Why do muscle cells have a large number of mitochondria?

9 Name two organelles that are similar to prokaryotic cells.

10 If plant cells have chloroplasts for photosynthesis, why do they also need mitochondria?

11 What is the importance of scale bars on micrographs?

To learn more about prokaryotic and eukaryotic cells, and the features of bacterial cells, go to the hotlinks site, search for the title or ISBN, and click on Chapter 1: Section 1.2.

1.3 Membrane structure

Understandings:

- Phospholipids form bilayers in water due to the amphipathic properties of phospholipid molecules.
- Membrane proteins are diverse in terms of structure, position in the membrane, and function.
- Cholesterol is a component of animal cell membranes.

Applications and skills:

- Application: Cholesterol in mammalian membranes reduces membrane fluidity and permeability to some solutes.
- Skill: Drawing of the fluid mosaic model.
- Skill: Analysis of evidence from electron microscopy that led to the proposal of the Davson–Danielli model.
- Skill: Analysis of the falsification of the Davson–Danielli model that led to the Singer–Nicolson model.

Guidance

- *Amphipathic phospholipids have hydrophilic and hydrophobic properties.*
- *Drawings of the fluid mosaic model of membrane structure can be two-dimensional rather than three-dimensional. Individual phospholipid molecules should be shown using the symbol of a circle with two parallel lines attached. A range of membrane proteins should be shown including glycoproteins.*

NATURE OF SCIENCE

Using models as representations of the real world: there are alternative models of membrane structure.

Falsification of theories, with one theory being superseded by another: evidence falsified the Davson–Danielli model.

Membrane structure

As early as 1915 scientists were aware that the structure of membranes isolated from cells included proteins and lipids. Further research established that the lipids were phospholipids. Early theories were mostly concerned with phospholipids forming a bilayer with proteins, forming thin layers on the exterior and interior of the bilayer. The Davson–Danielli model, proposed by Hugh Davson and James Danielli in 1935, used this lipid bilayer model, suggesting it was covered on both sides by a thin layer of globular protein.

In 1972, Seymour J. Singer and Garth L. Nicolson proposed that proteins are inserted into the phospholipid layer and do not form a layer on the phospholipid bilayer surfaces. They believed that the proteins formed a mosaic floating in a fluid layer of phospholipids. There were several reasons why Singer and Nicolson proposed a model that was different from the Davson–Danielli model. These reasons included the following.

- Not all membranes are identical or symmetrical, as the first model implied.
- Membranes with different functions also have a different composition and different structure, as can be seen with an electron microscope.
- A protein layer is not likely because it is largely non-polar and would not interface with water, as shown by cell studies.

Much of the evidence used to change the Davson–Danielli model was gathered with the use of the electron microscope. Another source of evidence was the study of cells and their actions in various environments and solutions. The ability to culture cells in the laboratory allowed many of these studies. Since 1972 further evidence has been gathered about membranes, and slight changes to the Singer–Nicolson model have been made.

TOK

Using models is a way in which scientists explain complex structures such as cellular membranes. Models are based on the knowledge available at the time a theory is suggested. Even though the early models of cell membranes were later proved wrong (because of new data), they helped in the development of the presently accepted model of cell membranes. Discuss why it is important to learn about theories that are later discredited.

The current agreed model for the cellular membrane is the fluid mosaic model. It is shown in the following diagram. All cellular membranes, whether plasma membranes or organelle membranes, have the same general structure.

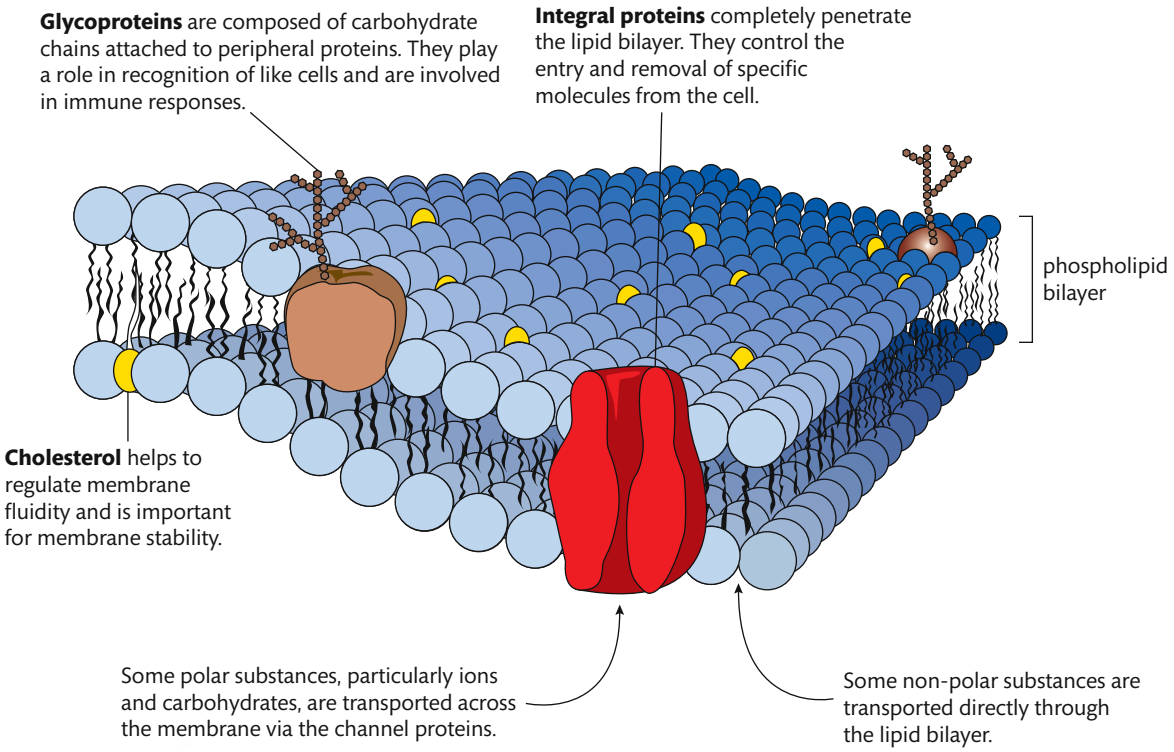
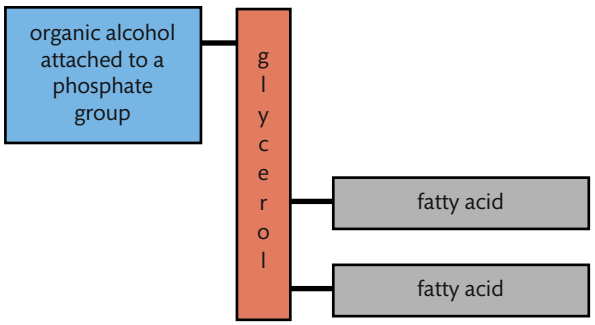


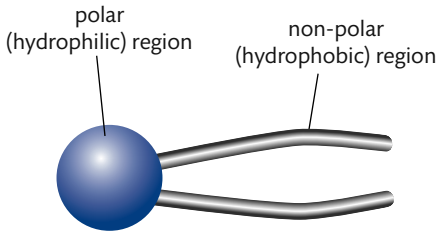
Figure 1.14 In the fluid mosaic model of the cell membrane there is a double layer of lipids (fats) arranged with their tails facing inwards. Proteins are thought to 'float' in the lipid bilayer.

Phospholipids

In Figure 1.14 note that the 'backbone' of the membrane is a bilayer produced from huge numbers of molecules called phospholipids. Each phospholipid is composed of a three-carbon compound called glycerol. Two of the glycerol carbons have fatty acids. The third carbon is attached to a highly polar organic alcohol that includes a bond to a phosphate group. Fatty acids are not water soluble because they are non-polar. In contrast, because the organic alcohol with phosphate is highly polar, it is water soluble. This structure means that membranes have two distinct areas when it comes to polarity and water solubility. One area is water soluble and polar, and is referred to as hydrophilic (water-loving). This is the phosphorylated alcohol side. The other area is not water soluble and is non-polar. It is referred to as hydrophobic (water-fearing).

Figure 1.15 This is a model of a phospholipid.





The hydrophobic and hydrophilic regions cause phospholipids to align as a bilayer if there is water present and there is a large number of phospholipid molecules. Because the fatty acid 'tails' do not attract each other strongly, the membrane tends to be fluid or flexible. This allows animal cells to have a variable shape and also allows the process of endocytosis (which is discussed below) to take place. What maintains the overall structure of the membrane is the tendency water has to form hydrogen bonds.

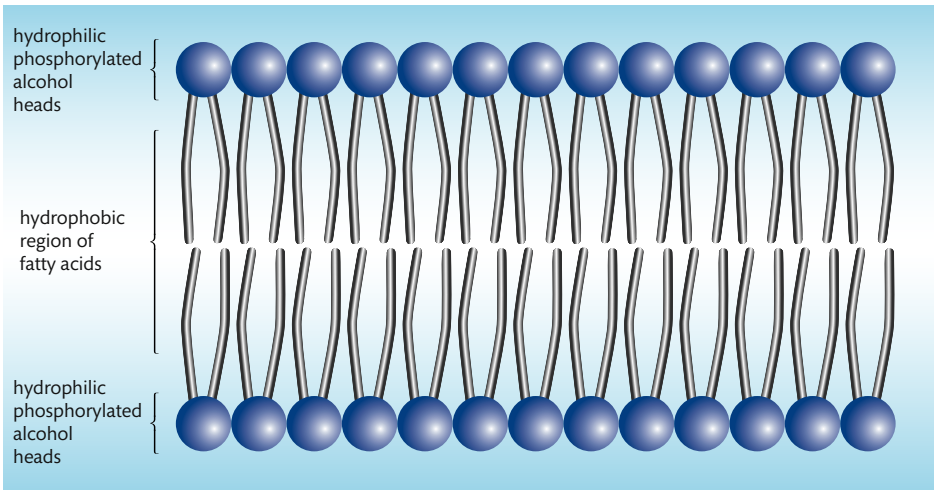


Figure 1.16 This model of a phopholipid bilayer shows how phospholipid molecules behave in two layers. Both layers have the phosphorylated alcohol end of the molecules towards the outside and the fatty acid tails oriented towards each other in the middle.

Cholesterol

Membranes must be fluid to function properly. They are a bit like olive oil in their consistency. At various locations in the hydrophobic region (fatty acid tails) in animal cells are cholesterol molecules. These molecules have a role in determining membrane fluidity, which changes with temperature. The cholesterol molecules allow membranes to function effectively at a wider range of temperatures than if they were not present. Plant cells do not have cholesterol molecules; they depend on saturated or unsaturated fatty acids to maintain proper membrane fluidity.

Proteins

The last major component of cellular membranes comprises the proteins. It is these proteins that create the extreme diversity in membrane function. Proteins of various types are embedded in the fluid matrix of the phospholipid bilayer. This creates the mosaic effect referred to in the fluid mosaic model. There are usually two major types of proteins. One type is referred to as integral proteins and the other type is referred to as peripheral proteins. Integral proteins show an amphipathic character, with both hydrophobic and hydrophilic regions within the same protein. These proteins will have the hydrophobic region in the mid-section of the phospholipid backbone. Their hydrophilic region will be exposed to the water solutions on either side of the membrane. Peripheral proteins, on the other hand, do not protrude into the middle hydrophobic region, but remain bound to the surface of the membrane. Often these peripheral proteins are anchored to an integral protein. Look at the drawing of the fluid mosaic model (Figure 1.14) to see the location of these proteins.

Make sure you can draw and label all the parts of a membrane as described in this section for the fluid mosaic model. Follow the directions given earlier for making a good drawing. In the drawing, the phospholipids should be shown using the symbol of a circle with two parallel lines attached. It is also important to show a wide range of proteins with various functions and locations.

Membrane protein functions

As you will recall, it is the membrane proteins that impart different functions to the different membranes. There are many different proteins, which have six general functions:

- sites for hormone-binding
- enzymatic action
- cell adhesion
- cell-to-cell communication
- channels for passive transport
- pumps for active transport.

Proteins that serve as hormone-binding sites have specific shapes exposed to the exterior that fit the shape of specific hormones. The attachment between the protein and the hormone causes a change in the shape of the protein, which results in a message being relayed to the interior of the cell.

Cells have enzymes attached to membranes that catalyse many chemical reactions. The enzymes may be on the interior or the exterior of the cell. Often they are grouped so that a sequence of metabolic reactions, called a metabolic pathway, can occur.

Cell adhesion is provided by proteins that can they hook together in various ways to provide permanent or temporary connections. These connections, referred to as junctions, can include gap junctions and tight junctions.

Many of the cell-to-cell communication proteins have carbohydrate molecules attached. They provide an identification label that represents the cells of different types of species.

Some proteins contain channels that span the membrane, providing passageways for substances to be transported through. When this transport is passive, material moves through the channel from an area of high concentration to an area of lower concentration.

In active transport, proteins shuttle a substance from one side of the membrane to another by changing shape. This process requires the expenditure of energy in the form of ATP. It does not require a difference in concentration to occur.

A passive process does not need the cell to provide any energy for it to occur. If energy is needed for a process to occur, that process is called active and the form of energy most often used is a type of nucleic acid called adenosine triphosphate or ATP.

Exercises

12 Explain the orientation of the bilayer of phospholipid molecules in the plasma membrane using the terms hydrophobic and hydrophilic.

13 Why does a diet high in plants and plant products have relatively low cholesterol levels compared with a diet involving high amounts of animal products?

14 What type of properties do amphipathic phospholipids possess?

15 What do many of the proteins of the plasma membrane involved with cell-to-cell communication have attached to them?

1.4 Membrane transport

Understandings:

- Particles move across membranes by simple diffusion, facilitated diffusion, osmosis, and active transport.
- The fluidity of membranes allows materials to be taken into cells by endocytosis or released by exocytosis. Vesicles move materials within cells.

Applications and skills:

- Application: Structure and function of sodium–potassium pumps for active transport and potassium channels for facilitated diffusion in axons.
- Application: Tissues or organs to be used in medical procedures must be bathed in a solution with the same osmolarity as the cytoplasm to prevent osmosis.
- Skill: Estimation of osmolarity in tissues by bathing samples in hypotonic and hypertonic solutions.

Guidance

- *Osmosis experiments are a useful opportunity to stress the need for accurate mass and volume measurements in scientific experiments.*

NATURE OF SCIENCE

Experimental design: accurate quantitative measurements in osmosis experiments are essential.

Passive and active transport

There are two general types of cellular transport:

- passive transport
- active transport.

As mentioned previously, passive transport does not require energy (in the form of ATP), but active transport does. Passive transport occurs in situations where there are areas of different concentrations of a particular substance. Movement of the substance occurs from an area of higher concentration to an area of lower concentration. Movement is said to occur along a concentration gradient.

When active transport occurs, the substance is moved against a concentration gradient, so energy expenditure must occur.

Passive transport: diffusion and osmosis

Examine Figure 1.17. It shows chemical diffusion.

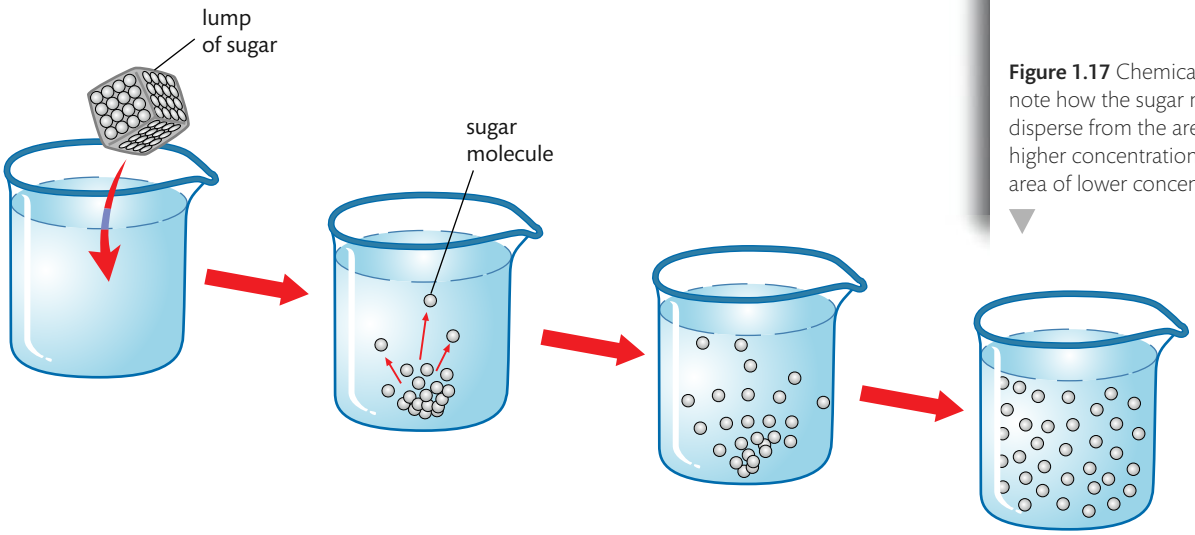


Figure 1.17 Chemical diffusion: note how the sugar molecules disperse from the area of higher concentration to the area of lower concentration.

Diffusion

Diffusion is one type of passive transport. Particles of a certain type move from a region of higher concentration to a region of lower concentration. However, in a living system, diffusion often involves a membrane. For example, oxygen gas moves from outside a cell to inside that cell. Oxygen is used by the cell when its mitochondria carry out respiration, thus creating a relatively lower oxygen concentration inside the cell compared with outside the cell. Oxygen diffuses into the cell as a result. Carbon dioxide diffuses in the opposite direction to the oxygen because carbon dioxide is produced as a result of mitochondrial respiration.

Facilitated diffusion

An example of a disease involving facilitated diffusion is cystinuria. This occurs when the protein that carries the amino acid cysteine is absent from kidney cells. The result is a build-up of amino acids in the kidney, resulting in very painful kidney stones.

Facilitated diffusion is a particular type of diffusion involving a membrane with specific carrier proteins that are capable of combining with the substance to aid its movement. The carrier protein changes shape to accomplish this task but does not require energy.

It should be evident from this explanation that facilitated diffusion is very specific depending on the carrier protein. The rate of facilitated diffusion will level off when total saturation of the available carriers occurs.

Osmosis

Partially permeable membranes are also called selectively permeable membranes.

Passive transport will occur until there is an equal concentration of the substance in both areas involved. This is called equilibrium.

Osmosis is another type of passive transport: movement occurs along a concentration gradient. However, osmosis involves only the passive movement of water across a partially permeable membrane. A partially permeable membrane is one that only allows certain substances to pass through (a permeable membrane would allow everything through). A concentration gradient of water that allows the movement to occur is the result of a difference between solute concentrations on either side of a partially permeable membrane. A hypertonic (hyperosmotic) solution has a higher concentration of total solutes than a hypotonic (hypo-osmotic) solution. Water therefore moves from a hypotonic solution to a hypertonic solution across a partially permeable membrane (study Figure 1.18). If isotonic solutions occur on either side of a partially permeable membrane, no net movement of water is evident.

An aid to remembering the difference between diffusion and osmosis is 'H_2Osmosis', linking the solvent water to osmosis.

Table 1.7 summarizes diffusion and osmosis (passive transport) across cellular membranes.

Table 1.7 Diffusion and osmosis

Type of passive transport	Description of membrane
Simple diffusion	Substances other than water move between phospholipid molecules or through proteins that possess channels
Facilitated diffusion	Non-channel protein carriers change shape to allow movement of substances other than water
Osmosis	Only water moves through the membrane using aquaporins, which are proteins with specialized channels for water movement

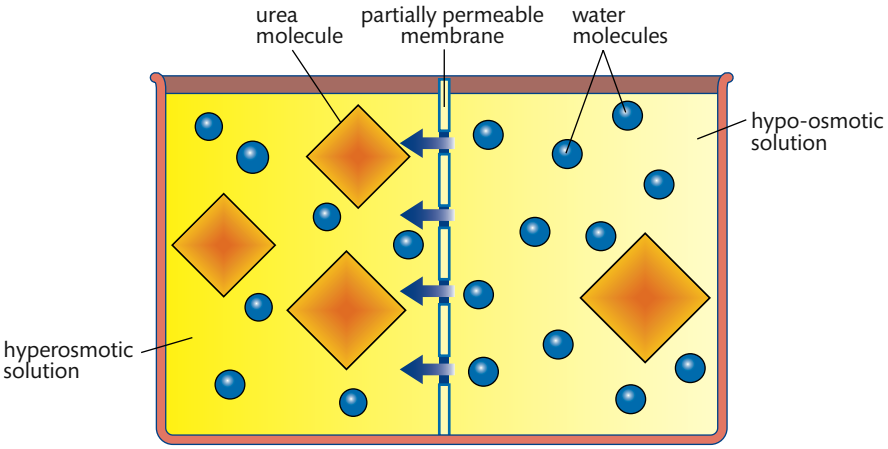


Figure 1.18 The partially permeable membrane between two solutions of different osmotic concentrations allows water molecules to pass from the hypo-osmotic solution to the hyperosmotic solution.

Size and charge

The size and polarity of molecules determine the ease with which various substances can cross membranes. These characteristics and the ability of molecules to cross membranes are arranged along a continuum like this:

small and non-polar molecules cross membranes easily ⟷ **large and polar molecules cross membranes with difficulty**

How easily a substance can move across a membrane passively depends on two major factors: size and charge. Substances that are small in size and non-polar will move across a membrane with ease. Substances that are polar, large in size, or both, do not cross membranes easily. Examples of small, non-polar substances are gases such as oxygen, carbon dioxide, and nitrogen. Ions such as chloride ions, potassium ions, and sodium ions have a great deal of difficulty crossing membranes passively, as do large molecules such as glucose and sucrose. Molecules such as water and glycerol are small, uncharged polar molecules that can cross membranes fairly easily.

A practical example of diffusion and osmosis is kidney dialysis. Many people have problems regulating blood solutes (solutes are substances that are dissolved in a solvent, in this case blood, to form a solution).

Solutions occur throughout the body in various types of spaces: intracellular spaces occur inside cells; extracellular spaces occur outside cells; interstitial spaces occur between cells; intravascular spaces occur within blood vessels.

Problems in regulating the solutes in the many body spaces can arise as the result of some sort of irregularity in the function of the kidneys. This can ultimately threaten a person's life because of the lack of homeostatic levels of solutes. To re-establish homeostasis, a process called haemodialysis may be carried out.

In this process, blood is passed through a system of tubes composed of selectively permeable membranes. These tubes are surrounded with a solution that is referred to as the dialysate. The dialysate contains key solutes at levels close to the patient's normal blood levels. Wastes are kept at a low level in the dialysate. As blood moves through the tubes, the dialysate is constantly replaced to maintain ideal levels.

CHALLENGE YOURSELF

Use your knowledge of osmosis, diffusion, membrane transport, and kidney dialysis to answer the following questions.

6 When solutes move from the blood through the selectively permeable membrane into the dialysate, what process is occurring?

7 Why is the process that allows wastes to move from the blood to the dialysate referred to as passive?

8 What is the importance of constantly changing the dialysate?

9 Name some characteristics of solutes in blood that would affect their rate of movement through the selectively permeable membrane.

10 Haemodialysis also allows regulation of water concentrations within the blood. What process is occurring when water moves through the tube membranes into the dialysate?

11 What factors might affect the time necessary for dialysis to bring about homeostatic blood levels of solutes and wastes?

Dialysis is also an example of how cell or tissue osmolarity (the concentration of osmotically active particles) can be estimated. If cells are placed in a solution of known osmolarity, there are three possibilities: the cells may gain mass, the cells may lose mass, or the cells may remain at the same mass. Answer the following questions.

12 If a group of cells are placed in a hypotonic solution, what will happen to their mass? Explain your answer.

13 One way to stop undesirable plants growing at a specific location is to apply a solution of water with a high concentration of sodium chloride (table salt). Why does this kill the plants and prevent their return for a period of time?

Determining the osmolarity of tissues

Safety alerts. Use safety goggles and lab aprons. Be cautious of cork borers and any other sharp instruments used. Wash your hands thoroughly with soap and water after each day's procedures.

Follow these instructions to determine the osmolarity of tissues by bathing samples in hypotonic, isotonic, and hypertonic solutions. The instructions use potatoes as a source of tissue, but other tissues could be used.

1. With a cork borer, cut six cores from a potato. The cores should all be as close to the same length as possible: 30–50-mm cores are recommended. Each core should be kept separate and identified as core A, core B, core C, core D, core E, and core F.
2. Before continuing, produce a table that will show the volume and mass of the potato cores before and after being placed in solutions of six different sucrose molarities. The molarities to be used are 0.0 M, 0.2 M, 0.4 M, 0.6 M, 0.8 M, and 1.0 M.
3. Using an appropriately sized graduated cylinder approximately one-half filled with water, determine the volume of each core using fluid displacement. Record this information.
4. Once each core is removed from the graduated cylinder, blot it dry with a paper towel and determine its mass using a laboratory balance. Record your results in the table.
5. Place each core in a different test tube labelled with the core's identification letter and the molarity of the sucrose solution to be placed in the tube.
6. Add a labelled molar solution to each test tube until the core is covered. Place foil or plastic wrap over each tube and store for 24 hours.
7. On the next day, repeat steps 3 and 4. Record your 24-hour results in the table.
8. Data processing
   - Produce a table to record the processed data involving the percentage change in mass and core volume.
   - Calculate the percentage change in mass, and the percentage change in volume, at the end of 24 hours, for each core. Record your results in the processed data table.
   - Construct an appropriate graph, with the independent variable of sucrose molarity on the *x*-axis and the dependent variable of mass percentage change on the *y*-axis.
   - Construct a similar graph showing volume percentage change.
9. Analysis
   - What is the osmolarity of the potato tissue? Explain how you determined this.
   - Explain the importance of accurate mass and volume measurements in this procedure, and in all scientific experiments.
   - Suggest some ways in which this procedure could be altered so that more reliable data could be attained.

Active transport and the cell

As you will remember, active transport requires work to be performed. This means energy must be used, so ATP is required. Active transport involves the movement of substances against a concentration gradient. This process allows a cell to maintain interior concentrations of molecules that are different from exterior concentrations.

Animal cells have a much higher concentration of potassium ions than their exterior environment, whereas sodium ions are more concentrated in the extracellular environment than in the cells. The cell maintains these conditions by pumping potassium ions into the cell and pumping sodium ions out of it. Along with energy, a membrane protein must be involved for this process to occur.

The sodium–potassium pump

The mechanism for actively moving sodium and potassium ions, the sodium–potassium pump, has five stages.

1 A specific protein binds to three intracellular sodium ions.

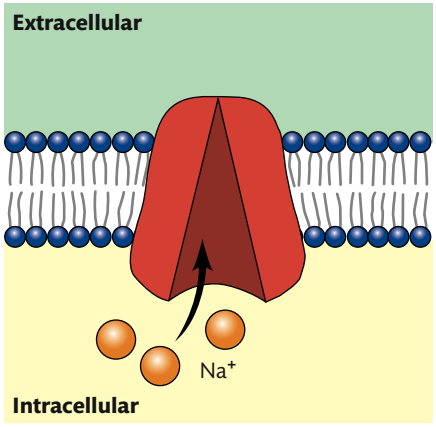


Figure 1.19 Stage 1: A protein in a phospholipid bilayer opens to the intracellular side and attaches three sodium ions.

2 The binding of sodium ions causes phosphorylation by ATP. ATP has three attached phosphates. When it carries out phosphorylation, one phosphate is lost resulting in a two-phosphate compound called ADP. ATP and ADP are discussed in more detail in Chapter 2.

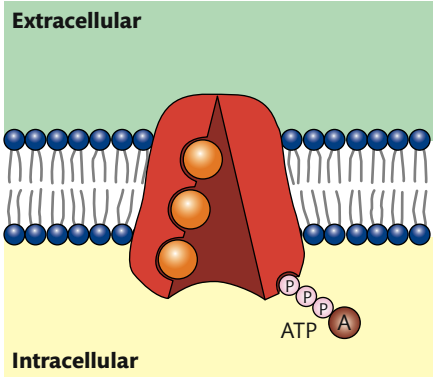


Figure 1.20 Stage 2: ATP attaches to the protein.

3 The phosphorylation causes the protein to change its shape, thus expelling sodium ions to the exterior.

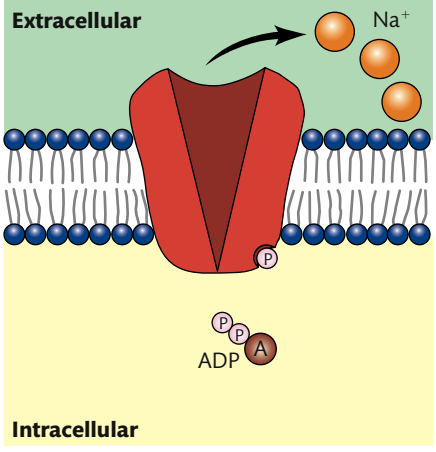


Figure 1.21 Stage 3: The carrier opens to the exterior of the cell and the sodium ions are released. ADP is released, leaving a phosphate group attached to the protein.

4 Two extracellular potassium ions bind to different regions of the protein, and this causes the release of the phosphate group.

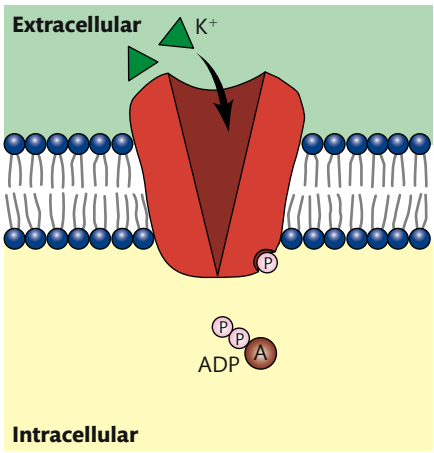


Figure 1.22 Stage 4: Extracellular potassium ions attach to the protein.

5 The loss of the phosphate group restores the protein's original shape, thus causing the release of the potassium ions into the intracellular space.

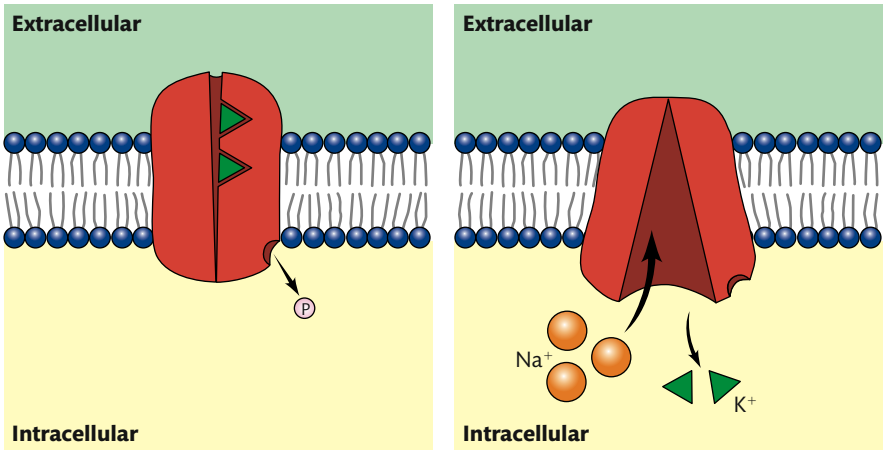


Figure 1.23 Stage 5: The protein opens towards the cell interior again and releases the potassium ions into the interior.

The sodium–potassium pump shows how important and active specific proteins are in the active transport of particular substances. It is also clear how ATP plays a crucial role in active transport.

There are many other examples of active transport in cells besides the sodium–potassium pump. Liver cells use active transport to accumulate glucose molecules from blood plasma even though the liver has a higher glucose concentration.

Cystic fibrosis is a human genetic disease in which the membrane protein that transports chloride ions is missing. This causes high concentrations of water inside the cells that line the lungs, and abnormally thickened mucus production. It is a very serious condition.

Endocytosis and exocytosis

Endocytosis and exocytosis are processes that allow larger molecules to move across the plasma membrane. Endocytosis allows macromolecules to enter the cell, while exocytosis allows molecules to leave. Both processes depend on the fluidity of the plasma membrane. It is important to recall why the cell membranes are fluid in consistency: the phospholipid molecules are not closely packed together, largely because of the rather 'loose' connections between the fatty acid tails. It is also important to remember why the membrane is quite stable: the hydrophilic and hydrophobic properties of the different regions of the phospholipid molecules cause them to form a stable bilayer in an aqueous environment.

Endocytosis occurs when a portion of the plasma membrane is pinched off to enclose macromolecules or particulates. This pinching off involves a change in the shape of the membrane. The result is the formation of a vesicle that then enters the cytoplasm of the cell. The ends of the membrane reattach because of the hydrophobic and

hydrophilic properties of the phospholipids and the presence of water. This could not occur if the plasma membrane did not have a fluid nature.

Exocytosis is essentially the reverse of endocytosis, so the fluidity of the plasma membrane and the hydrophobic and hydrophilic properties of its molecules are just as important as in endocytosis. One example of cell exocytosis involves proteins produced in the cytoplasm of a cell. Protein exocytosis usually begins in the ribosomes of rough ER and progresses through a series of four steps, outlined below, until the substance produced is secreted to the environment outside the cell.

1. Protein produced by the ribosomes of the rough ER enters the lumen, inner space, of the ER.
2. Protein exits the ER and enters the *cis* side or face of the Golgi apparatus; a vesicle is involved.
3. As the protein moves through the Golgi apparatus, it is modified and exits on the *trans* face inside a vesicle.
4. The vesicle with the modified protein inside moves to and fuses with the plasma membrane; this results in the secretion of the contents from the cell.

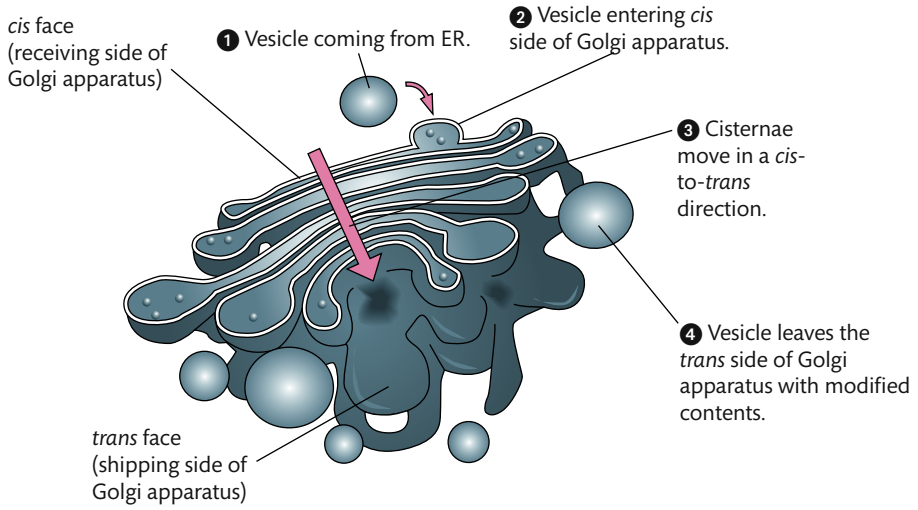


Figure 1.24 How the Golgi apparatus functions.

The fluidity of the plasma membrane is essential to allow fusion and subsequent secretion of the vesicle contents. At this point the vesicle membrane is actually a part of the plasma membrane.

Examples of endocytosis include:

- **phagocytosis, the intake of large particulate matter**
- **pinocytosis, the intake of extracellular fluids.**

Examples of exocytosis occur when:

- **pancreas cells produce insulin and secrete it into the bloodstream (to help regulate blood glucose levels)**
- **neurotransmitters are released at synapses in the nervous system.**

Exercises

16 Why is the term equilibrium used with passive but not active transport?

17 What type of amino acids will be present where integral proteins attach to cell membranes?

18 Why are exocytosis and endocytosis known as examples of active transport?

NATURE OF SCIENCE

Testing the general principles that underlie the natural world: the principle that cells only come from pre-existing cells needs to be verified.

1.5 The origin of cells

Understandings:

- Cells can only be formed by division of pre-existing cells.
- The first cells must have arisen from non-living material.
- The origin of eukaryotic cells can be explained by the endosymbiotic theory.

Applications and skills:

- Application: Evidence from Pasteur's experiments that spontaneous generation of cells and organisms does not now occur on Earth.

Guidance

- *Evidence for the endosymbiotic theory is expected. The origin of eukaryote cilia and flagella does not need to be included.*
- *Students should be aware that the 64 codons in the genetic code have the same meanings in nearly all organisms, but that there are some minor variations that are likely to have accrued since the common origin of life on Earth.*

Cell theory

The cell theory was discussed in Section 1.1. We mentioned that the current theory has three main parts:

1. all organisms are composed of one or more cells
2. cells are the smallest units of life
3. all cells come from other pre-existing cells.

We also mentioned that there are some problems with and exceptions to the current cell theory. These exceptions will now be discussed. Scientists use the term theory to represent a well-substantiated explanation of a natural phenomenon that incorporates tested hypotheses and laws. Because of this, a theory is an extremely valuable endpoint of science that represents understandings that have developed from extensive observation, experimentation, and logical inferences. Cell theory is a prime example of this. It has been modified during the years since it was first proposed in the 1800s. It will continue to be modified as cellular research progresses in the future.

One obvious missing component of cell theory is how the first cell arose. There is no evidence that new cells arise from non-living material today. However, the first cells must have been formed in this way. As already mentioned, in the 19th century the famous French scientist Louis Pasteur showed that bacteria could not spontaneously appear in sterile broth. Here is an overview of his experiment.

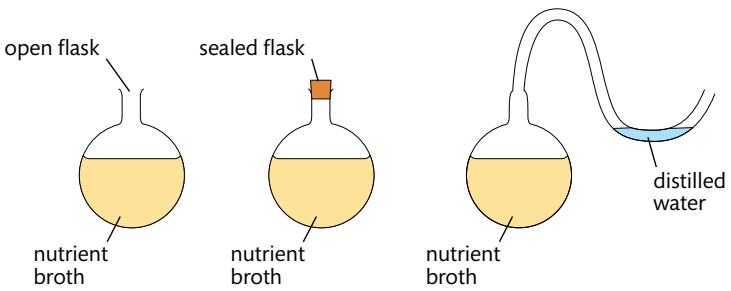


Figure 1.25 Pasteur's broth experiment.

1. He boiled a nutrient broth.
2. The now sterile nutrient broth was then placed in three flasks, as shown to the left. Incubation over a period of time was then allowed.
3. A sample of each flask was then transferred to a plate containing solid medium and incubated.

The only flask sample that showed the presence of bacteria was the opened one. The other two did not show any bacterial growth. This indicated to Pasteur that the concept of spontaneous generation was wrong.

The Italian scientist Francesco Redi also questioned the concept of spontaneous generation, nearly 200 years before Pasteur, and conducted an experiment using raw meat in jars. Many other experiments have been done since the work of these two science pioneers, casting further doubt on the idea of spontaneous generation.

Moving on to the exceptions to the current cell theory, these include:

- the multinucleated cells of striated muscle cells, fungal hyphae, and several types of giant algae
- very large cells with continuous cytoplasm that are not compartmentalized into separate smaller cells
- viruses
- the problem of explaining the 'first' cells without spontaneous generation.

These examples represent exceptions to the 'normal' cells that we see in most of the organisms on Earth today. Continued research is needed to see how these exceptions 'fit' in with the current cell theory.

A common origin for all cells on Earth requires an explanation of how a cell could progress from a simple, non-compartmentalized prokaryote to a complex, highly compartmentalized eukaryote. This is currently explained by the endosymbiotic theory. This theory was presented by Lynn Margulis in 1981. Key points of the theory include:

- about 2 billion years ago a bacterial cell took up residence inside a eukaryotic cell
- the eukaryotic cell acted as a 'predator', bringing the bacterial cell inside
- the eukaryotic cell and the bacterial cell formed a symbiotic relationship, in which both organisms lived in contact with one another
- the bacterial cell then went through a series of changes to ultimately become a mitochondrion.

In this process, the eukaryote helped the bacteria by providing protection and carbon compounds. The bacteria, after a series of changes, became specialized in providing the eukaryote with ATP. There is a lot of evidence to support this theory. Mitochondria:

- are about the size of most bacterial cells
- divide by fission, as do most bacterial cells
- divide independently of the host cell
- have their own ribosomes, which allows them to produce their own proteins
- have their own DNA, which more closely resembles the DNA of prokaryotic cells than of eukaryotic cells
- have two membranes on their exterior, which is consistent with an engulfing process.

In addition to the mitochondria, chloroplasts in plant cells also provide evidence for the theory of endosymbiosis. A modern-day protist called *Hatena* normally fulfils its nutritional needs by ingesting organic matter. However, when it behaves as a predator and ingests a green alga, it switches its method of fulfilling its nutritional needs to one that uses sunlight to convert organic molecules, a process known as photosynthesis. The two organisms, the *Hatena* and the green alga, continue to thrive in a symbiotic relationship.

Plasmodial slime moulds, of the phylum Myxomycota, are composed of eukaryotic cells. They are found in forests as a single large cell formed when many individual motile cells fuse. These cells have many nuclei and may increase in size to several centimetres. They are even capable of slow but coordinated movement. These organisms certainly present a different image of a cell than usually thought of when discussing the cell theory.

NATURE OF SCIENCE

Now is an ideal time to have a classroom discussion about cell characteristics, the functions of life, and exceptions to the current cell theory. In the discussion be open to the ideas of others, remembering that this is a key characteristic of a successful scientist.

CHALLENGE YOURSELF

14 On a sheet of paper produce a series of drawings that represent how two membranes could have come to exist on mitochondria and chloroplasts through an engulfing process involving endocytosis.

Biology is concerned not only with life, but also anything that affects life. When studying organisms, it is possible to take two approaches. One approach, often referred to as reductionism, reduces the complex phenomena of organisms to the interaction of their parts. Essentially, this viewpoint says it is the sum of the parts that make up the complex system, the organism. Another approach is that of holism or of looking at systems. This approach has the central belief that the whole is greater than the sum of its parts. Discuss how both approaches have allowed the accumulation of the body of knowledge we now possess in biology. Attempt to utilize both approaches to explain the functions of life as demonstrated by a single cell.

Another organism, *Elysia chlorotica*, demonstrates a similar situation. *Elysia* is a slug found in salt and tidal marshes and creeks. Its early stage of life, referred to as its juvenile stage, characteristically involves movement and it derives its nutrition by ingesting nutrients from its surroundings. During this juvenile stage it is brown. As it develops, if *Elysia* comes into contact with a specific type of green algae, it will enter its adult phase, in which chloroplasts from the ingested algae will be retained in its digestive tract. The adult stage of *Elysia* is therefore green in colour. The symbiotic relationship between *Elysia* and the green algae allows the adult form of *Elysia* to take on a more sedentary lifestyle, depending on light being available to carry out photosynthesis.

The final bit of evidence for endosymbiotic theory is DNA. DNA provides a code made up of 64 different 'words'. Interestingly, this code has the same meaning in nearly all organisms on Earth and is said to be 'universal'. There are only slight variations, which can be explained by changes since the common origin of life on our planet. As mentioned above, the mitochondria of eukaryotic cells have a DNA code that more closely resembles bacteria than eukaryotic cells. Most scientists believe that the more DNA two organisms have in common, the more closely related they are to one another.

Exercises

19 Why did bacteria grow in the broth of the flask that was left open by Pasteur?

20 Provide an explanation for how a nucleus might have come to exist within eukaryotic cells.

21 How does the example of *Hatena* and the alga represent an emergent explanation of life?

22 From the evidence presented in this section, explain why many scientists feel there has been an unbroken chain of life from the first cells on Earth to all cells in organisms alive today.

NATURE OF SCIENCE

Serendipity and scientific discoveries: the discovery of cyclins was accidental.

1.6 Cell division

Understandings:

- Mitosis is division of the nucleus into two genetically identical daughter nuclei.
- Chromosomes condense by supercoiling during mitosis.
- Cytokinesis occurs after mitosis and is different in plant and animal cells.
- Interphase is a very active phase of the cell cycle with many processes occurring in the nucleus and cytoplasm.
- Cyclins are involved in the control of the cell cycle.
- Mutagens, oncogenes, and metastasis are involved in the development of primary and secondary tumours.

Applications and skills:

- Application: The correlation between smoking and incidence of cancers.
- Skill: Identification of phases of mitosis in cells viewed with a microscope or in a micrograph.
- Skill: Determination of a mitotic index from a micrograph.

Guidance

- *The sequence of events in the four phases of mitosis should be known.*
- *Preparation of temporary mounts of root squashes is recommended but phases in mitosis can also be viewed using permanent slides.*
- *To avoid confusion in terminology, teachers are encouraged to refer to the two parts of a chromosome as sister chromatids, while they are attached to each other by a centromere in the early stages of mitosis. From anaphase onwards, when sister chromatids have separated to form individual structures, they should be referred to as chromosomes.*

The cell cycle

The cell cycle describes the behaviour of cells as they grow and divide. In most cases, the cell produces two cells that are genetically identical to the original. These are called daughter cells. The cell cycle integrates a growth phase with a divisional phase. Sometimes, cells multiply so rapidly that they may form a solid mass of cells called a tumour. We refer to this disease state as cancer. It appears that any cell can lose its usual orderly pattern of division, because we have found cancer in almost all tissues and organs.

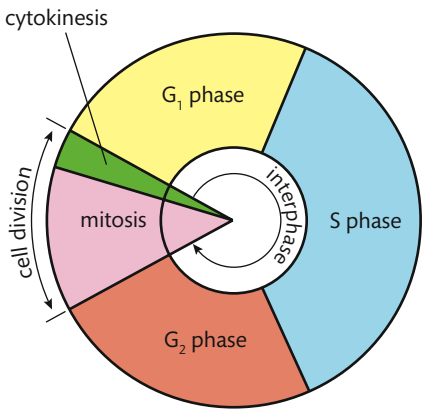


Figure 1.26 The cell cycle in eukaryotes.

You may wonder what causes a cell to go out of control. To answer this question, we must first understand the ordinary cell cycle. Usually, the life of a cell involves two major phases. In one phase, growth is the major process. In the other phase, division is the major process. The cell cycle begins and ends as one cell, so it can be represented by a circle divided into various named sections, as shown in Figure 1.26.

Interphase

The largest phase of the cell cycle in most cells is interphase. This is the longest and most variable of the cell-cycle phases. Interphase includes three smaller phases: G_1, S, and G_2. During G_1, the major event is growth of the cell. At the beginning of G_1, the cell is the smallest it will ever be. After G_1 comes the S phase, in which the main activity is replication of the DNA of the cell, the chromosomes. This phase is sometimes referred to as the synthesis phase. Once the chromosomes have been replicated, the cell enters its second growth phase, called G_2. During this phase, the cell grows and makes preparations for mitosis, the M phase. During G_2, organelles may increase in number, DNA begins to condense from chromatin to chromosomes, and microtubules may begin to form.

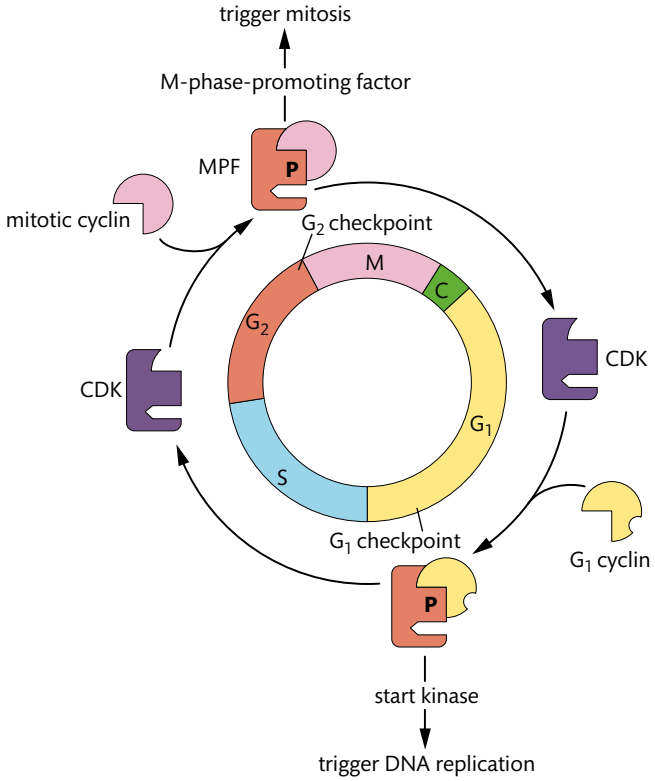


Figure 1.27 Two cyclins are extremely important to the cell cycle: G_1 cyclin and mitotic cyclin. Note their location in the cell cycle and that they must combine with a CDK to become active.

Interphase is a very active period in a cell's life. It involves metabolic reactions, DNA replication, and an increase in the number of organelles. Because interphase involves growth, it is essential that protein synthesis occurs at a rapid rate during this phase.

NATURE OF SCIENCE

Tim Hunt and Joan Ruderman were studying gene expression in early embryos. While doing so they found three proteins that varied in concentrations at different times of the cell cycle. These were eventually called cyclins. This illustrates that scientists must always be observant, to spot unplanned and surprising discoveries. This example of 'accidental' discovery is common in science.

Cyclins are a group of proteins that control the cell's progression through the cell cycle. The cyclins bind to cyclin-dependent protein kinases (CDKs), enabling them to act as enzymes. These activated enzymes then cause the cell to move from G_1 to the S phase and from G_2 to the M phase. The points where the cyclin-activated CDKs function are called checkpoints in the cell cycle. Some cells will pause during G_1 and enter a separate phase, the G_0 phase. G_0 is a non-growing state and certain cells stay in G_0 for varying periods of time. Some cells, such as nerve and muscle cells, never progress beyond the G_0 phase.

Mitosis

To remember the correct order of phases in the cell cycle and mitosis, remember the word 'shipmate'. If you take away the word 'she', you get 'ipmat': these letters give you the order of interphase, prophase, metaphase, anaphase, and telophase.

Once all the preparatory processes have taken place, and the DNA has replicated, the cell moves into mitosis or the M phase. During mitosis the replicated chromosomes separate and move to opposite poles of the cell, thus providing the same genetic material at each of these locations. When the chromosomes are at the poles of the cell, the cytoplasm divides to form two cells distinct from the larger parent. These two cells have the same genetic material and are referred to as daughter cells.

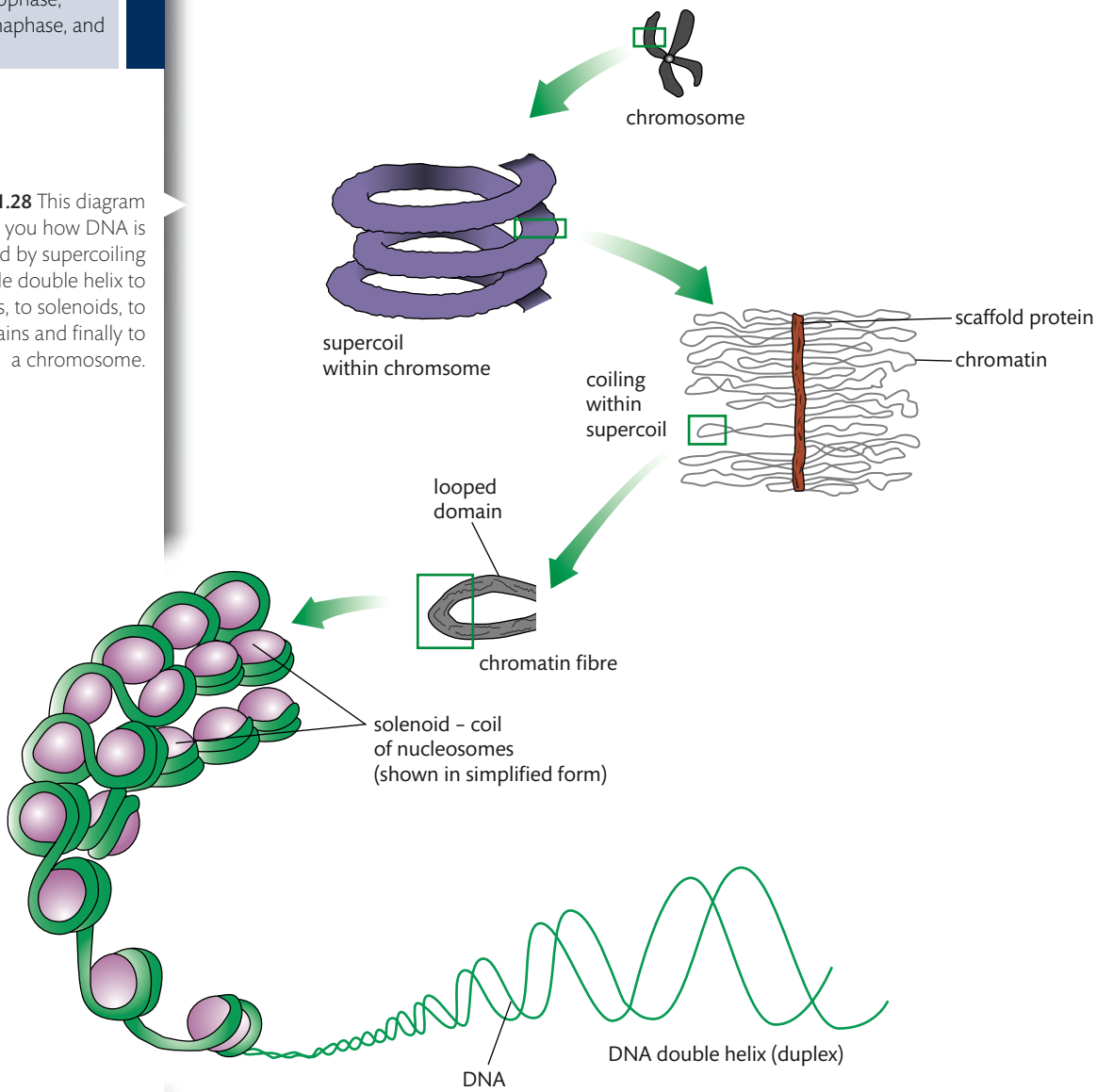


Figure 1.28 This diagram shows you how DNA is packaged by supercoiling from a single double helix to nucleosomes, to solenoids, to looped domains and finally to a chromosome.

Mitosis involves four phases. They are, in sequence:

- prophase
- metaphase
- anaphase
- telophase.

Before considering a detailed description of these phases, it is essential you understand the chromosome. As you will recall, during the second growth phase, G_2, the chromatin (elongated DNA and histones) begins to condense. This condensation is accomplished via a process called supercoiling. First, the DNA wraps around histones to produce nucleosomes. The nucleosomes are further wrapped into a solenoid. Solenoids group together in looped domains, and then a final coiling occurs to produce the chromosome.

Eukaryotic cells contain chromosomes that, before replication in the S phase of the cell cycle, are composed of one molecule of DNA. After replication, the chromosome includes two molecules of DNA. These two identical molecules are held together by the centromere, and each molecule is referred to as a chromatid. Together, they are called sister chromatids. The chromatids will eventually separate during the process of mitosis. When they do, each is then called a chromosome and each has its own centromere.

Once you are familiar with the structure of a chromosome, you can understand the four phases of mitosis. Remember, when a cell enters the phases of mitosis, replication of DNA has already occurred. Therefore, the chromosomes at this stage are each composed of two sister chromatids.

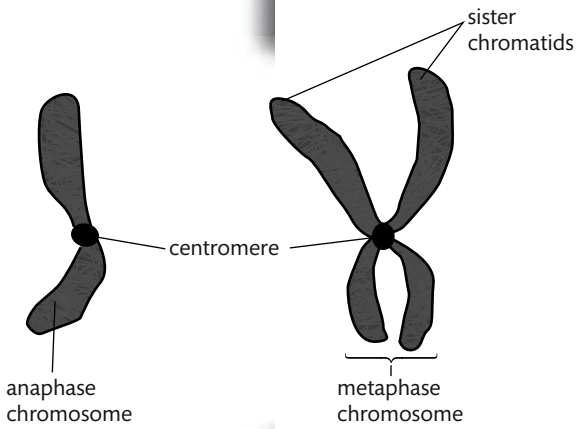


Figure 1.29 An anaphase chromosome is a single molecule of DNA and has a centromere. A metaphase chromosome has sister chromatids attached at the centromere.

Prophase

Examine the figure below.

1. The chromatin fibres become more tightly coiled to form chromosomes.
2. The nuclear envelope disintegrates and nucleoli disappear.
3. The mitotic spindle begins to form and is complete at the end of prophase.
4. The centromere of each chromosome has a region called the kinetochore that attaches to the spindle.
5. The centrosomes move towards the opposite poles of the cell as a result of lengthening microtubules.

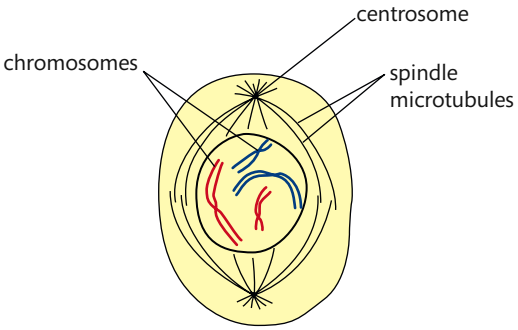


Figure 1.30 This animal cell is in prophase. For clarity, only a small number of chromosomes is shown.

Metaphase

Examine the figure below.

1. The chromosomes move to the middle or equator of the cell. This is referred to as the metaphase plate.
2. The chromosomes' centromeres lie on the plate.
3. The movement of chromosomes arises as the result of the action of the spindle, which is made of microtubules.

4 The centrosomes are now at the opposite poles.

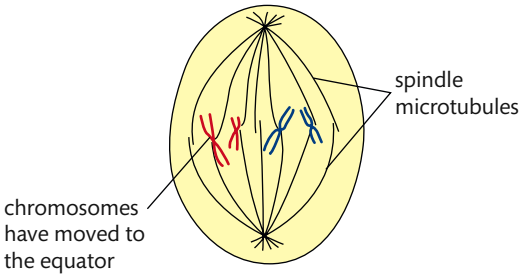


Figure 1.31 The cell is now in metaphase. Again, only a small number of chromosomes is shown.

Anaphase

Examine the figure below.

1 This is usually the shortest phase of mitosis. It begins when the two sister chromatids of each chromosome are split.
2 These chromatids, now chromosomes, move towards the opposite poles of the cell.
3 The chromatid movement arises as a result of the shortening of the microtubules of the spindle.
4 Because the centromeres are attached to the microtubules, they move towards the poles first.
5 At the end of this phase, each pole of the cell has a complete, identical set of chromosomes.

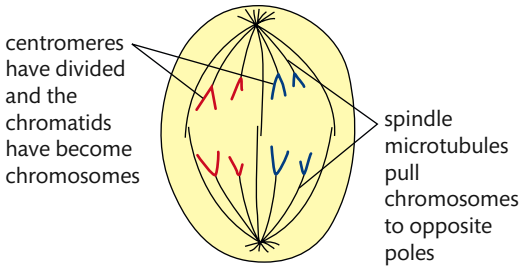


Figure 1.32 The cell is now in anaphase. Again, only a small number of chromosomes is shown.

Telophase

Examine the figure below.

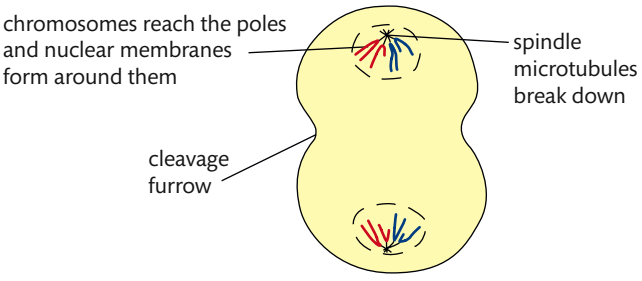


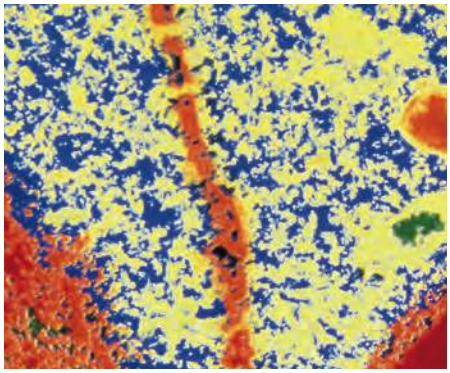
Figure 1.33 Finally, the cell enters telophase.

1 The chromosomes are at each pole.
2 A nuclear membrane (envelope) begins to re-form around each set of chromosomes.
3 The chromosomes start to elongate to form chromatin.
4 Nucleoli reappear.
5 The spindle apparatus disappears.
6 The cell is elongated and ready for cytokinesis.

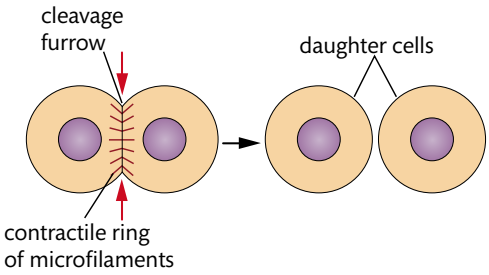
Cytokinesis

As you can see, the phases of mitosis involve nuclear division. It appears that the process of mitosis occurs in discrete stages. But this is not in fact true: the stages occur along a continuum. We only use the separate stages to help us understand the overall process.

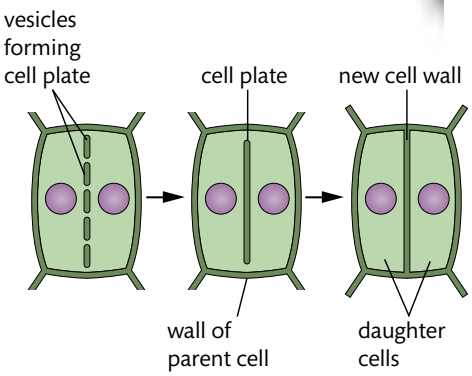
Once nuclear division has occurred, the cell undergoes cytokinesis. Cytokinesis in animal cells involves an inward pinching of the fluid plasma membrane to form cleavage furrows. However, plant cells have a relatively firm cell wall and they form a cell plate. The cell plate occurs midway between the two poles of the cell and moves outwards towards the sides of the cell from a central region. Both processes result in two separate daughter cells that have genetically identical nuclei.



Left:
Frog embryo showing cells dividing to form the 4-cell stage.
Right:
Micrograph showing the telophase stage of mitosis in the root tip cell of maize. Most noticeable is the cell wall being formed that will separate the daughter cells in cytokinesis.



Cleavage of an animal cell (SEM)

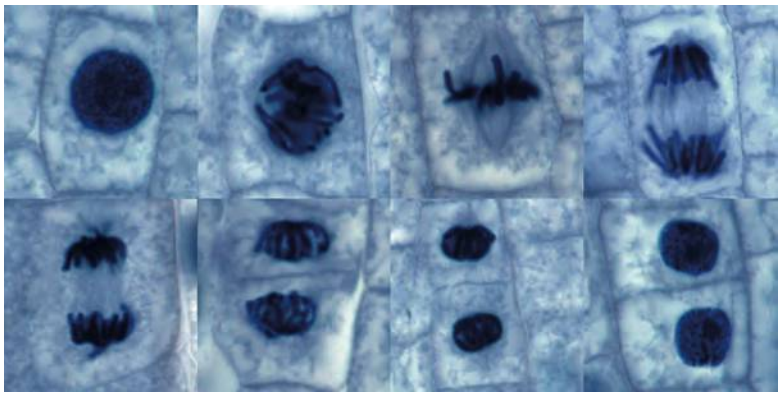


Cell plate formation in a plant cell (TEM)

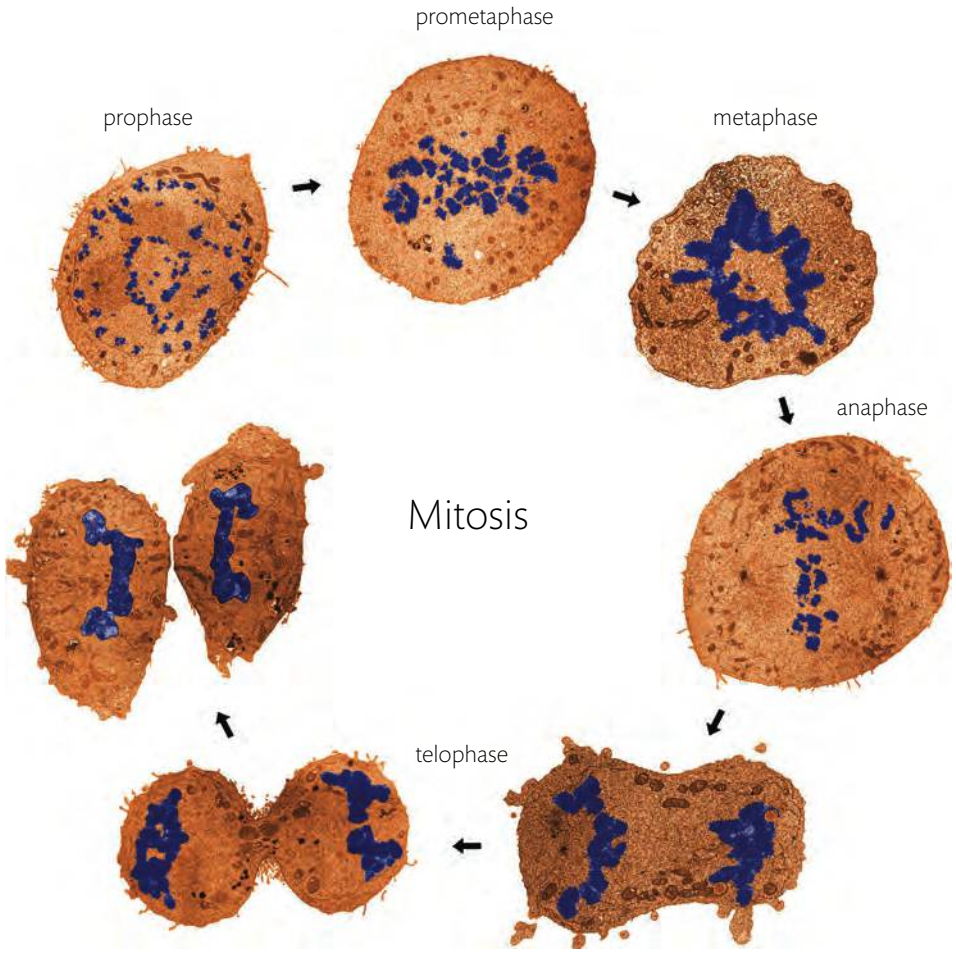
Figure 1.34 Cytokinesis in animal and plant cells.

The growth of organisms, development of embryos, tissue repair, and asexual reproduction all involve mitosis. Mitosis does not happen by itself. It is a part of the cell cycle.

Study the micrographs on page 46 to note the main events of the stages of mitosis and to note the differences between plant and animal cells in mitosis. Note that A is from a plant cell and B is from an animal cell.



A Micrograph of root tip cells from an onion undergoing mitosis. From top left to bottom right: the chromosomes condense and appear as long thread-like structures (prophase). They then align along the centre of the cell (metaphase). Each chromosome consists of two identical sister chromatids that separate and are pulled to opposite ends of the cell (anaphase). Nuclear membranes then form around the two daughter nuclei as the chromosomes de-condense (telophase). The cell then divides (cytokinesis).



B Composite of transmission electron micrographs showing the stages of mitosis in a human lymphocyte.

Laboratories all over the world are busy researching causes and treatments for all known types of cancers. Sharing of information is a common occurrence amongst these laboratories and their researchers.

Cancer

As mentioned earlier, cancer occurs when a cell's cycle becomes out of control. The result is a mass of abnormal cells referred to as a tumour. A primary tumour is one that occurs at the original site of a cancer. A secondary tumour is a metastasis, a cancerous tumour that has spread from the original location to another part of the organism. An example of metastasis is a brain tumour that is in fact composed of breast cancer cells. In some cases the metastasis of the primary tumour cells is so extensive that secondary tumours are found in many locations within the organism.

The mitotic index is an important tool for predicting the response of cancer cells to chemotherapy. It is the ratio of the number of cells in a tumour or tissue type undergoing mitosis compared with the number of cells not undergoing mitosis. A higher mitotic index indicates a more rapid proliferation of cells of a certain type. It is likely that tumours with higher mitotic indices will be more difficult to control, and a patient with such a tumour may be given a poorer prognosis than a patient with a tumour that has a lower mitotic index.

A question to consider here is how or why a primary tumour forms. Most organisms have sections of genes that may mutate or may be expressed at abnormally high levels. These sections of genes, called oncogenes, contribute to converting a normal cell into a cancer cell. The oncogenes may start to change or go through mutation because they are triggered by an outside agent referred to as a mutagen. One such potential mutagen is cigarette smoke. There is a correlation between smoking and the incidence of cancer. This has been shown consistently in many independent studies. Examine the graph from the World Health Organization below and note the positive correlation.

TOK This is an appropriate time to discuss the unethical actions of the tobacco industry in the suppression of results linking smoking with cancer. A global perspective should be included in this discussion.

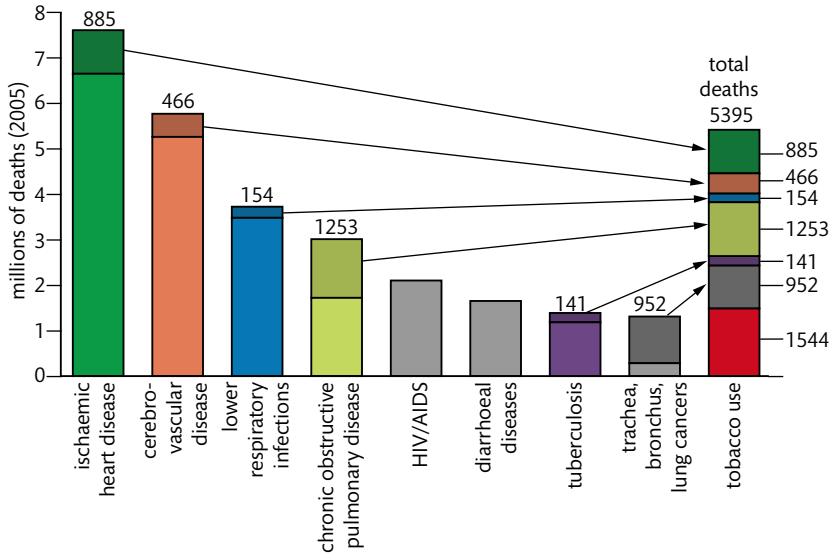


Figure 1.35 Examine this graph from the World Health Organization noting tobacco use as a risk factor for eight leading causes of death in the world. Especially note the information provided concerning trachea, bronchus, and lung cancers. Darker areas indicate proportions of deaths that are related to tobacco use and are coloured according to the column of the respective cause of death. WHO, http://www.who.int/tobacco/mpower/graphs/en/index.html

A very interesting activity is to find micrographs on the internet of various human tissues going through cell division. Determine the mitotic index of these various types of tissue. Attempt to find micrographs of the same types of tissue that are cancerous and determine their mitotic index. This comparison will show quite effectively the more rapid cell division found in cancer tissues.

CHALLENGE YOURSELF

Cancer cells have a higher rate of mitotic division than normal cells. Because of this, cancer tissue has a higher mitotic index than normal tissue. This is why cancer cells can grow and spread very rapidly.

15 If a microscopic field of 1000 normal cells has 900 cells in interphase, estimate the number of cells in interphase when 1000 cells of the same type are from tissue that is cancerous.

16 If the normal cells have an average cell cycle time of 600 minutes, estimate the average, relative cell cycle time of the cancer cells.

17 How does this information affect the mitotic index of the two sets of cells?

To learn more about the mitotic phases, go to the hotlinks site, search for the title or ISBN, and click on Chapter 1: Section 1.6.

Exercises

23 A chemical called colchicine disrupts the formation of microtubules. What effect would this drug have on a cell going through mitosis?

24 If a parent cell has 24 chromosomes, how many chromatids would be present during metaphase of mitosis?

25 Explain when cytokinesis occurs within the cell cycle.

26 Compare cytokinesis in plant and animal cells.

27 What is the value of the mitotic index?

Practice questions

1 The micrograph below shows an adult human stem cell.

(a) The cell cycle can be divided into two parts: interphase and mitosis.

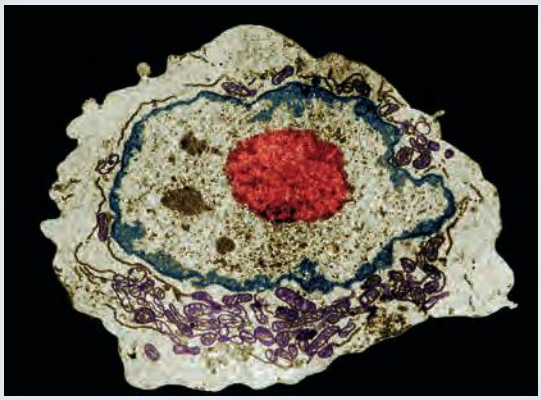
(i) Identify, with a reason, whether the stem cell in the micrograph is in interphase or mitosis. (1)

(ii) Deduce **two** processes that occur in human cells during this part of the cell cycle, but not during the other part. (2)

(b) State **two** characteristics of stem cells that can be used to distinguish them from other body cells. (2)

(c) Outline **one** therapeutic use of stem cells. (3)

(*Total 8 marks*)

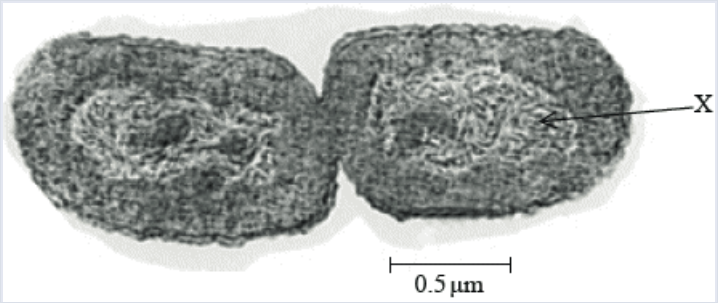


2 Below is a micrograph of an *E. coli* bacterium undergoing reproduction.

In the diagram what does label X identify?

A Nucleoid region **B** Chromatin **C** Histones **D** Endoplasmic reticulum

(*Total 1 mark*)



www.bio.mtu.edu/campbell/prokaryo.htm

3 Which of the following is **not** a function performed by a membrane protein?

A Hormone-binding sites **B** Cell adhesion **C** Enzyme synthesis **D** Pumps for active transport

(*Total 1 mark*)

4 Which of the following take(s) place during either interphase or mitosis in animal cells?

I. Re-formation of nuclear membranes

II. Pairing of homologous chromosomes

III. DNA replication

A I only **B** I and II only **C** II and III only **D** I and III only

(*Total 1 mark*)

5 (a) The scanning electron micrograph below shows the surface of the nuclear envelope with numerous nuclear pores.

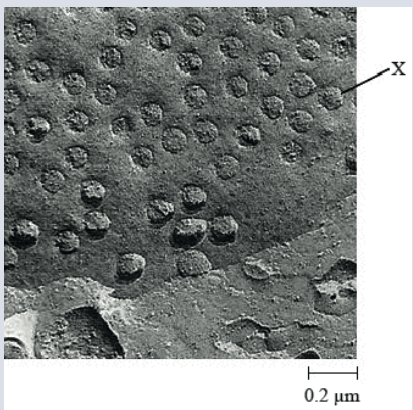
(i) Calculate the power of magnification of the image. (1)

(ii) State the diameter of the pore labelled X. (1)

(b) List **two** examples of how human life depends on mitosis. (1)

(c) Describe the importance of stem cells in differentiation. (3)

(*Total 6 marks*)



Adapted from Nelson and Cox 2000, © Don W. Fawcett/ Science Source

02 Molecular biology

Computer image of an insulin molecule. Insulin is a peptide (protein) hormone that helps to regulate glucose levels between the bloodstream and the cytoplasm of cells.

Essential ideas

2.1	Living organisms control their composition by a complex web of chemical reactions.
2.2	Water is the medium of life.
2.3	Compounds of carbon, hydrogen, and oxygen are used to supply and store energy.
2.4	Proteins have a very wide range of functions in living organisms.
2.5	Enzymes control the metabolism of the cell.
2.6	The structure of DNA allows efficient storage of genetic information.
2.7	Genetic information in DNA can be accurately copied and can be translated to make the proteins needed by the cell.
2.8	Cell respiration supplies energy for the functions of life.
2.9	Photosynthesis uses the energy in sunlight to produce the chemical energy needed for life.

Organic chemistry is the chemistry of carbon compounds. Biochemistry is the branch of organic chemistry that attempts to explain the chemistry characteristics of living organisms. Even though biochemistry can be amazingly complex and varied, there are common patterns that are well known. For example, all living organisms are made up of molecules that can be classified into one of four types:

- carbohydrates
- lipids
- proteins
- nucleic acids.

In addition, biochemical processes in living organisms follow certain common pathways for which we can study the common pattern. So, when we study cell respiration or photosynthesis as biochemical processes, we do not need to study a completely different process for each organism or species.

This chapter will introduce you to some of the more common biochemically important molecules and processes.

2.1 Molecules to metabolism

Understandings:

- Molecular biology explains living processes in terms of the chemical substances involved.
- Carbon atoms can form four covalent bonds, allowing a diversity of stable compounds to exist.
- Life is based on carbon compounds, including carbohydrates, lipids, proteins, and nucleic acids.
- Metabolism is the web of all the enzyme-catalysed reactions in a cell or organism.
- Anabolism is the synthesis of complex molecules from simpler molecules, including the formation of macromolecules from monomers by condensation reactions.
- Catabolism is the breakdown of complex molecules into simpler molecules including the hydrolysis of macromolecules into monomers.

NATURE OF SCIENCE

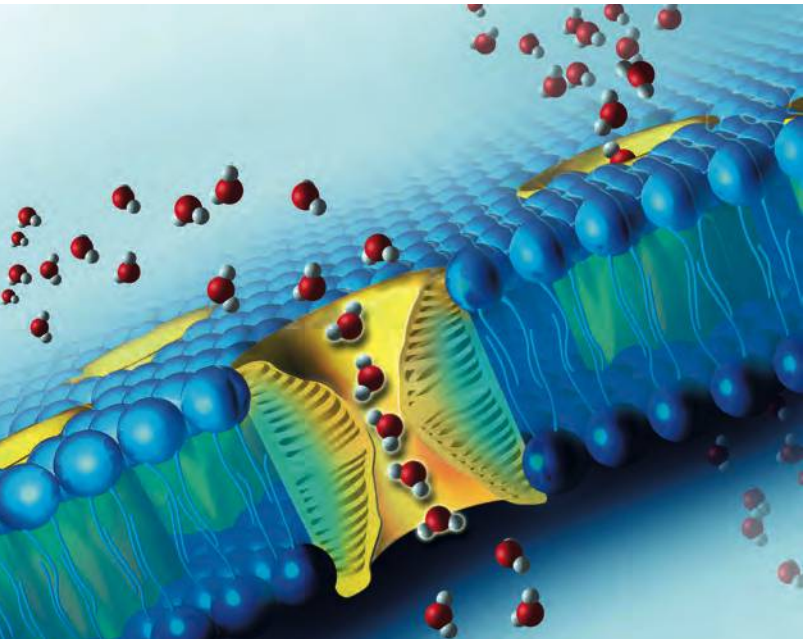
Falsification of theories: the artificial synthesis of urea helped to falsify vitalism.

Applications and skills:

- Application: Urea as an example of a compound that is produced by living organisms but can also be artificially synthesized.
- Skill: Drawing molecular diagrams of glucose, ribose, a saturated fatty acid, and a generalized amino acid.
- Skill: Identification of biochemicals such as sugars, lipids, or amino acids from molecular diagrams.

Guidance

- *Only the ring forms of D-ribose, alpha-D-glucose, and beta-D-glucose are expected in drawings.*
- *Sugars include monosaccharides and disaccharides.*
- *Only one saturated fat is expected, and its specific name is not necessary.*
- *The variable radical of amino acids can be shown as R. The structure of individual R-groups does not need to be memorized.*
- *Students should be able to recognize from molecular diagrams that triglycerides, phospholipids, and steroids are lipids. Drawings of steroids are not expected.*
- *Proteins or parts of polypeptides should be recognized from molecular diagrams showing amino acids linked by peptide bonds.*



Aquaporins are channels that allow water molecules to pass through the membrane.

Molecular biology is the chemistry of living organisms

The majority of molecules within all living organisms can be categorized into one of four biochemical groupings. Those groupings are carbohydrates, lipids, proteins, and nucleic acids. In turn, these four groupings of molecules interact with each other in a wide variety of ways in order to carry out the metabolism of each cell.

Consider the following example of metabolism in order to see how living processes are actually chemical substances interacting in predictable patterns. Insulin is a protein hormone that facilitates the movement of glucose from the bloodstream to the interior of cells. Insulin does this by interacting with protein channels in body cell plasma membranes, thereby opening those channels to glucose. As long as glucose is in a higher concentration outside the cell compared with inside the cell, glucose will continue to move through the open channel by diffusion. The plasma membrane is largely composed of a type of lipid called a phospholipid. Because of molecular polarity differences, phospholipids will not allow glucose to pass through the membrane without going through the protein channels. Both insulin and the channels within the plasma membrane are proteins, therefore they must both be coded for by deoxyribonucleic acid (DNA) within the cells of the organism in which they are working.

Glucose is a carbohydrate, the phospholipid molecules are lipids, both insulin and the membrane channels are proteins, and DNA is a nucleic acid. Each molecule has a specific function and collectively they all work together in order to ensure that body cells have access to glucose for their energy needs. All the biochemistry within all living organisms can be 'broken down' into smaller interactions similar to the above example.

CHALLENGE YOURSELF

1 Read through this example of molecular interactions leading to a physiological response. After doing so, try to classify each of the *named* molecular components as a carbohydrate, lipid, protein, or nucleic acid.
When a predator, such as a snake, catches and eats a small rodent, one of the main sources of nutrition that the snake is consuming is the muscle of the prey animal. That muscle is primarily composed of two molecules: actin and myosin. When the ingested muscle reaches the intestines of the snake, enzymes (such as trypsin) help the snake digest the actin and myosin into amino acids. Other enzymes (such as lipase) help the snake digest the triglyceride fats within the adipose tissue of the rodent.

Carbon-based life

Carbon dioxide is one of the very few carbon-containing substances that is not classified as organic. In this model the black, centre atom is the carbon atom, and the two red atoms are the oxygen atoms.

Organic chemistry is the study of compounds that contain carbon. Some compounds that contain carbon are not classified as organic, including carbon dioxide. Despite this important exception, there are very many molecules containing carbon that are classified as organic. The molecules already mentioned above (carbohydrates, proteins, lipids, and nucleic acids) are all organic molecules. These are the molecules from which all living things are composed, thus the element carbon can be considered to be the keystone element for life on Earth. This is the reason why you sometimes hear life on Earth being described as 'carbon based'.

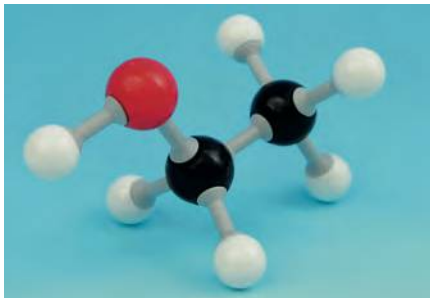
Carbon's name is derived from the Latin word 'carbo', meaning charcoal.

You may recall from your introductory chemistry course that each carbon atom has an atomic number of six. Directly this means that carbon has six protons, but indirectly it also means that carbon has six electrons. Two of these six electrons form the stable inner shell, and four are found in the second and unfilled shell. Carbon's way of 'filling' this second shell of electrons is to share four electrons with other atoms in order to create a stable configuration of eight electrons in total. Each time carbon shares one of its electrons, a covalent bond is formed, and carbon always forms four covalent bonds.

TOK You will notice that virtually all the images you see of atoms and molecules are in the form of models. Why are models used? What do the real atoms and molecules look like?

There are many other elements found within the molecules of living organisms. In addition to carbon, the following elements are common: hydrogen, oxygen, nitrogen, and phosphorus. These elements are used in the molecular structures of carbohydrates, proteins, lipids, and nucleic acids by forming covalent bonds with carbon, and very often by forming covalent bonds with each other.

The structure and bonding of an ethanol molecule. The two atoms shown in black are carbon, the red atom is oxygen, and all the white atoms are hydrogen.

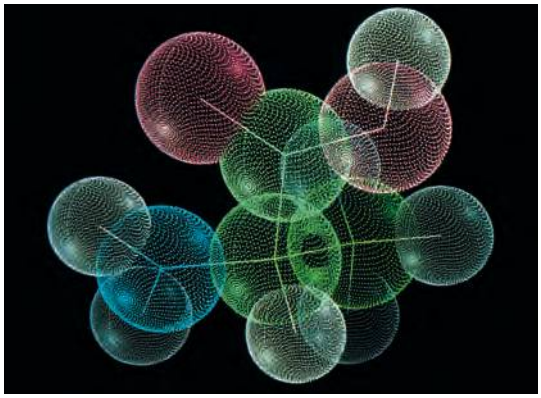


Biochemical compounds that are important to living organisms

Living things are composed of an amazing array of molecules. We can start to make sense of all of these molecules by classifying them into different types. Molecules of the same type have certain qualities in common and become fairly easy to recognize with a little practice. Table 2.1 shows some of the more common biochemically important molecules and their subcomponents (or building blocks).

Table 2.1 Types of molecules

Molecule	Subcomponents (building blocks)
Carbohydrates	Monosaccharides
Lipids	Glycerol, fatty acids, phosphate groups
Proteins (polypeptides)	Amino acids
Nucleic acids	Nucleotides



This is a colour-coded molecular model of the amino acid alanine. Green = carbon; pink = oxygen; blue = nitrogen; white = hydrogen

As you study biochemistry, you will soon learn to recognize and classify common biochemical molecules into appropriate categories. Table 2.2 shows some of the common categories and examples of molecules.

Table 2.2 Common categories of molecules

Category	Subcategory	Example molecules
Carbohydrates	Monosaccharides	Glucose, galactose, fructose, ribose
	Disaccharides	Maltose, lactose, sucrose
	Polysaccharides	Starch, glycogen, cellulose, chitin
Proteins		Enzymes, antibodies, peptide hormones
Lipids	Triglycerides	Fat stored in adipose cells
	Phospholipids	Lipids forming a bilayer in cell membranes
	Steroids	Some hormones
Nucleic acids		Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), adenosine triphosphate (ATP)

The ways in which these molecules interact with each other in living organisms is amazingly diverse and interesting. All of these interactions are referred to as metabolism and that is the focus of the next section.

Metabolism: reactions controlled by enzymes

If you were to visualize zooming into the inside of a cell down to the molecular level, you would see thousands of molecules colliding with each other as they move through their aqueous (water-based) environment. Many of these collisions do not result in

any action other than the molecules changing direction and thus heading into new collisions. But sometimes these molecular collisions provide enough energy to specific molecules, the reactants, for those reactants to undergo a chemical reaction of some type. That single chemical reaction would be one of the millions of reactions that occur within that cell that comprise that cell's metabolism. In a multicellular organism, all of the reactions within all of the cells (and fluids such as blood) comprise the metabolism of the organism.

When two molecules collide, there are a large number of factors that determine whether a reaction occurs or not. Some of these factors include the:

- identity of the colliding molecules
- orientation of the colliding molecules (where they hit each other)
- the speed of the molecules when they collide.

Cells use enzymes in order to increase the likelihood that a collision will lead to a useful reaction. Enzymes are protein molecules that have a specific shape into which a reactant(s) can fit, at a molecular location called the active site of the enzyme. By having an active site, the enzyme increases the likelihood of a reaction.

Let's look at an example of one reaction that makes up part of a typical cell's metabolism. The reaction we will consider is one in which adenosine triphosphate (ATP) is formed or synthesized. ATP is the most common molecule used by cells when chemical energy is required. ATP is synthesized from the bonding of adenosine diphosphate (ADP) to a phosphate (P) group. This reaction requires energy, and that energy may come originally from food (cell respiration) or sunlight (photosynthesis). Put simply, the reaction can be summarized as:

$$ADP + P_i \rightarrow ATP$$

adenosine diphosphate plus inorganic phosphate yields adenosine triphosphate

The odds of these two reactants (ADP + P_i) colliding at a very high speed, at exactly the correct orientation, leading to a new covalent bond forming between them, is extremely small. That is where an enzyme comes into play: the enzyme acts as a catalyst for the reaction. The catalyst will not be used up and so the enzyme will be available to act as a catalyst many times over. The ADP reactant fits into part of the enzyme's active site, and the inorganic phosphate group reactant fits perfectly oriented next to it, and, within a small fraction of a second, the two reactants become covalently bonded to each other. So, in effect, three molecules are involved in the collision but only two of them result in the production of ATP. The ATP is then released from the active site and the enzyme is ready for another collision with another ADP and phosphate group. The catalysis provided by the enzyme enables this reaction to occur at a much higher reaction rate and with less collisional energy compared with the same reaction occurring without the enzyme.

All of your metabolism is based on this fundamental scenario. A multitude of reactions are occurring inside each living organism's cells at any given moment. Most of these reactions are being catalysed by enzymes. These are the reactions that make up your overall metabolism, and include diverse sets of reactions, including:

- replication of DNA, in preparation for cell division
- synthesis of RNA, allowing chemical communication between the nucleus and cytoplasm

Metabolism is best thought about from a molecular perspective. Often, people think only of physiological parameters, such as heart rate and digestion, as their metabolism. But remember that metabolism is all of the reactions within all of the cells of an organism.

The 'collisional energy' referred to in this section is called activation energy. An enzyme is often defined as an organic catalyst that lowers the activation energy of a reaction.

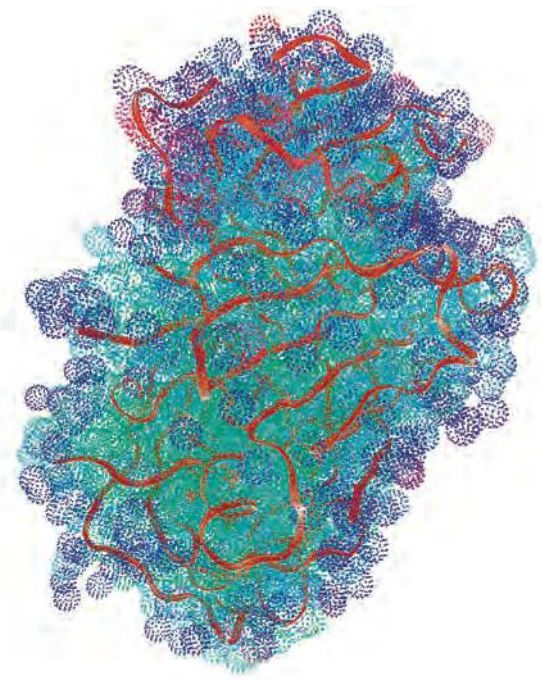
- synthesis of proteins, including bonding of one amino acid to another
- cell respiration, with nutrients being converted into ATP
- photosynthesis, with light energy being used to create carbohydrates
- and many, many more.

Many of the carbon atoms found in the food that you eat (such as carbohydrates) will be eliminated from your body in the molecules of carbon dioxide that you breathe out.

Metabolism = catabolism + anabolism

It is very common for people to use some form of the word metabolism in everyday conversations, for example: 'I wish I had a higher metabolic rate so that I could eat more without putting on weight'. When people say something like that, they are usually thinking of factors like their heart rate. There is actually a great deal more than this involved in metabolism. As described in the previous section, your metabolism is the sum total of all the enzyme-catalysed reactions taking place within you. Some of these enzyme-catalysed reactions function to convert large, complex molecules (like many of the foods that we eat) to smaller, simpler molecular forms. This is called catabolism. Other enzyme-catalysed reactions carry out the reverse: they convert small, simple molecules into a larger, more complex molecules. This is called anabolism. These molecular conversions are done for a variety of reasons, and we will look at a couple of examples in this section. You will find more examples later as you study the various biochemical and physiological processes common to living organisms.

Many organisms, including all animals, rely on the foods that they eat to obtain the building block molecules that make up their larger molecules. When animals eat foods, the food is digested (or hydrolysed) into the building blocks (catabolism). After these building blocks are transported to body cells, they are bonded together to form larger molecules once again (anabolism).



This computer graphic image shows pepsin, an enzyme that helps to digest proteins. Pepsin is an example of a hydrolysing enzyme.

Let's explore what happens to ingested foods. Foods are chemically digested in your alimentary canal. The digestive enzymes that accomplish this are hydrolysing

enzymes. Each reaction is called a hydrolysis and requires a molecule of water as a reactant. This is a good way to recognize hydrolysis reactions: water is always 'split' as part of the reaction. Below are four examples of hydrolysis reactions.

1 Hydrolysis of a disaccharide to two monosaccharides (see Figure 2.1).

lactose + water → glucose + galactose

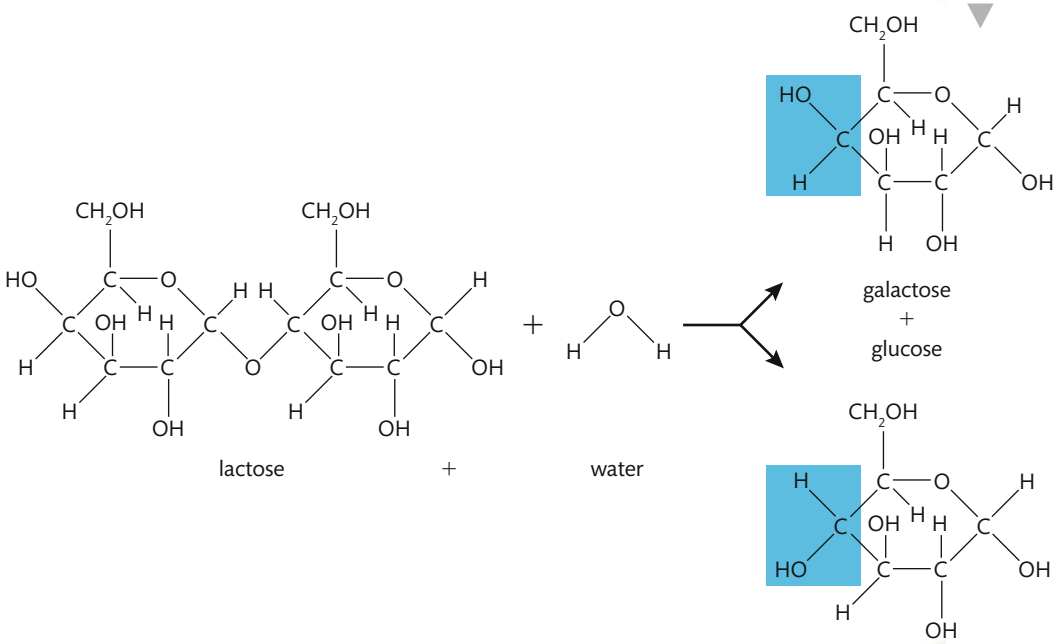


Figure 2.1 Hydrolysis of the disaccharide lactose to form the two monosaccharides galactose and glucose. The difference between galactose and glucose is shown in the blue areas.

2 Hydrolysis of a polysaccharide to many monosaccharides.

starch + (many) water → (many) glucose

3 Hydrolysis of a triglyceride lipid to glycerol and fatty acids (see Figure 2.2).

triglyceride + 3 water → glycerol + 3 fatty acids

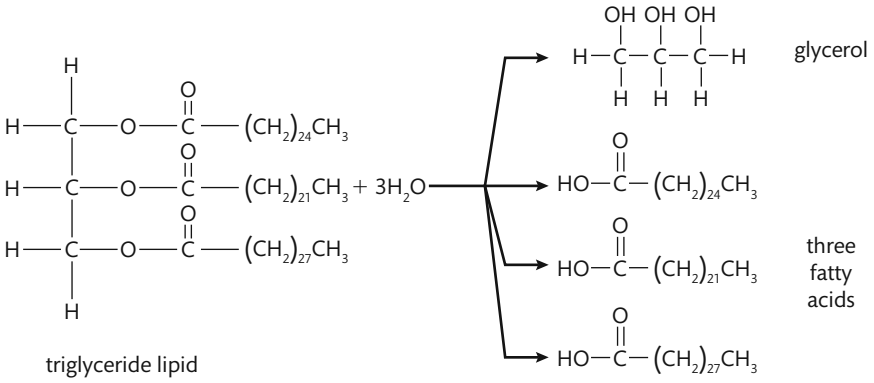


Figure 2.2 Hydrolysis of a triglyceride lipid to form glycerol and three fatty acid molecules.

4 Hydrolysis of a polypeptide (protein) to amino acids.

protein + (many) water → (many) amino acids

Condensation reactions are, in many ways, the reverse of hydrolysis reactions. In cells, condensation reactions occur to re-form the larger, biochemically important, molecules. In the four examples given above, simply reverse the reaction arrow and each example shows a condensation reaction. For example:

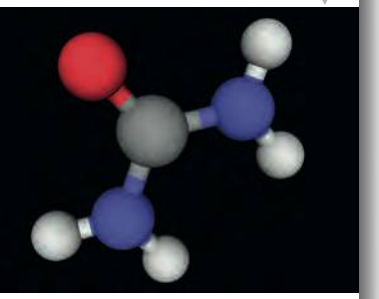
- condensation of amino acids to form a polypeptide

(many) amino acids → protein + (many) water

Notice that in condensation reactions, water molecule(s) are products rather than reactants. Condensation reactions require a different type of enzyme, one that is capable of catalysing reactions in which covalent bonds are created rather than broken.

In summary, remember that an organism's metabolism comprises all of the reactions that occur within all of its cells. Thus metabolism can also be thought of as the sum of all the reactions that work to hydrolyse large biochemical substances into smaller subcomponents (catabolism), plus all those reactions that rebuild large, more complex biochemical substances from the smaller subcomponents (anabolism).

Molecular model of urea. The large grey atom is carbon. Each of the two blue side-chains is an amine functional group and the red atom is a double-bonded oxygen atom.



The German physician and chemist Friedrich Wöhler.

NATURE OF SCIENCE

It is difficult for people growing up and learning in today's world to truly appreciate the scientific ideas of the past. One of the philosophies that was widely held nearly two centuries ago was called vitalism. Vitalism was the belief that living organisms and inanimate things differed fundamentally because living organisms contained a non-physical or vitalistic element, and were subject to different principles of nature compared with non-living things. A part of this philosophy even suggested that the organic molecules that are characteristic of living organisms could only be produced within living organisms.

One example of an organic molecule is urea. Urea is produced in some living organisms as a nitrogenous waste product. In mammals, including humans, urea is produced in the liver, enters the bloodstream, and is then filtered out of the bloodstream by the kidneys, and becomes a component of urine. The fundamentals of this process were known in the early 1800s, and it was assumed, because of the widely held principle of vitalism, that this was the only way urea could be produced.

In 1828, Friedrich Wöhler, a German physician and chemist, made a discovery that helped change the thinking behind vitalism. In his laboratory, Wöhler had mixed two inorganic substances, cyanic acid and ammonium, in a beaker. He noticed the formation of a crystalline substance that looked familiar to him. After testing, he confirmed that the crystals were urea. He had previously only come across urea crystals in the study of the compounds that are characteristic of urine. For perhaps the first time in a controlled setting, an organic molecule was synthesized from inorganic substances.

Wöhler did not fully appreciate the meaning and consequences of his findings at the time, but, as it turned out, his published work was soon used as evidence that vitalism should be questioned as a scientific theory. It was not long before other substances, such as amino acids, were synthesized from inorganic precursors in various laboratories.

What does this show about the nature of science?

- Scientific theories undergo modifications over time. Some are just modified, while some are proved to be completely false.
- Frequently, important discoveries are made 'accidently'. Dr Wöhler did not add the two inorganic substances together with the intention of making urea.
- Frequently, a scientific discovery is not appreciated immediately for its importance. This is one of the reasons why discoveries need to be published. This allows the entire scientific community to fit new knowledge into the bigger picture of science, and sometimes that only happens much later.

CHALLENGE YOURSELF

2 Drawing molecular diagrams of common biochemical substances is easier than you might think, especially with a little practice. You will be expected to be able to draw the following molecules from memory:

- alpha-D-glucose
- beta-D-glucose
- ribose
- an unnamed saturated fatty acid
- a generalized amino acid.

When drawing these, and other complex organic molecules, it helps to draw them in a sequential pattern. That sequence is given below.

(a) Draw the carbons first (this is called the carbon backbone of the molecule).
(b) Then add in any functional groups that are found as part of the molecule.
(c) 'Fill in' with hydrogen atoms, to ensure that all the carbon atoms are showing four covalent bonds.
(d) Look over your entire structure, to make sure that all of the different atom types are showing the correct number of covalent bonds for that type of element.
(e) If you know or have been given the chemical formula of the substance, count the number of each type of atom and check that number against the known formula.

Here is how it would work for the monosaccharide sugar called alpha-D-glucose, a substance that we know has the chemical formula of $C_6H_{12}O_6$.

(a)

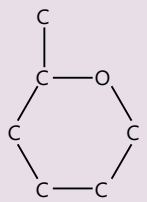


Figure 2.3 The carbon backbone of alpha-D-glucose.

(b)

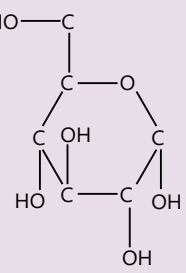


Figure 2.4 The alcohol groups added.

(c)

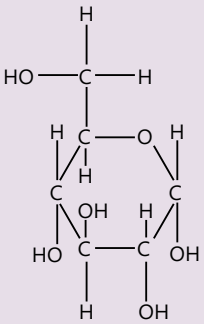


Figure 2.5 The hydrogens added.

Note: Be sure to count the covalent bonds around each element, and make sure that the number is appropriate for each. You should also count the number of each type of atom and check that against the known formula of $C_6H_{12}O_6$.

Beta-D-glucose has exactly the same chemical formula as alpha-D-glucose and the two are, in fact, isomers of each other. Alpha-D-glucose and beta-D-glucose differ only in how a few of the atoms within the structure are oriented in space in relation to each other. Here is the finished molecular diagram of beta-D-glucose:

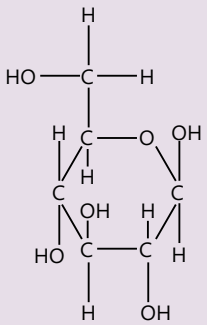


Figure 2.6 Beta-D-glucose.

Here are the completed molecular diagrams of another three molecules that you need to learn to draw from memory. Get out some paper and a pencil and practise drawing the two glucose molecules shown on the previous page and the three molecules shown below. Don't practise drawing them in their entirety, but use the step-by-step process as shown above. Do this until you are confident that you know each one very well.

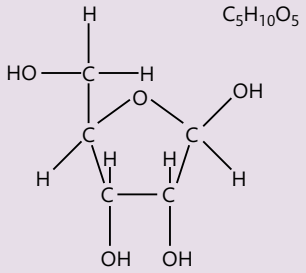


Figure 2.7 D-ribose.

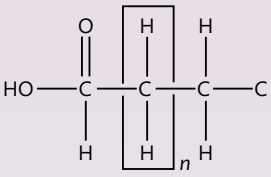


Figure 2.8 A generalized fatty acid.

Where *n* = any number between 3 and 29 (11–23 are the most common)

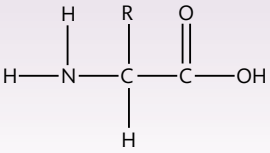


Figure 2.9 A generalized amino acid.

Where R = 1 of 20 variable groups

Trying to draw complex organic molecules by somehow memorizing the entire intact structure is frustrating and impossible for most people. Instead, always use the sequence of steps shown on the previous page, of laying out the carbon backbone, adding the functional groups, and then filling in with hydrogen(s) as needed. This will not only help you learn the molecules you need to know, but it will also enable you to look at large, complex biochemical molecules from a new and more useful perspective.

Exercises

1 One way to check whether organic molecules are drawn correctly is to make a sketch based on the information given and then count the number of atoms of each element using a given or known formula. Draw each of the molecules described below and then check each against the formula given in the answers.

(a) Sketch a single carbon atom, add an alcohol group, fill in with hydrogen atoms. Give the formula of the molecule.

(b) Sketch a single carbon atom, add an amine group, add a carboxyl group, fill in with hydrogens. Give the formula of the molecule.

2 Give the products of each of the following reactions:

(a) the complete hydrolysis of a starch molecule

(b) the condensation reaction between glucose and galactose

(c) the complete hydrolysis of a triglyceride lipid.

3 Briefly describe the two aspects of metabolism.

2.2 Water

Understandings:

- Water molecules are polar and hydrogen bonds form between them.
- Hydrogen bonding and dipolarity explain the cohesive, adhesive, thermal, and solvent properties of water.
- Substances can be hydrophilic or hydrophobic.

Applications and skills:

- Application: Comparison of the thermal properties of water with those of methane.
- Application: Use of water as a coolant in sweat.
- Application: Modes of transport of glucose, amino acids, cholesterol, fats, oxygen, and sodium chloride in blood in relation to their solubility in water.

Guidance

- *Students should know at least one example of a benefit to living organisms of each property of water.*
- *Transparency of water and maximum density at 4°C do not need to be included.*
- *Comparison of the thermal properties of water and methane assists in the understanding of the significance of hydrogen bonding in water.*

NATURE OF SCIENCE

Use theories to explain natural phenomena: the theory that hydrogen bonds form between water molecules explains the properties of water.

The structure of water molecules and the resulting polarity

Water is the solvent of life. Living cells typically exist in an environment in which there is water within the cell (cytoplasm) and also water in the surrounding environment (intercellular fluid, fresh or salt water, etc.). We refer to all solutions as aqueous solutions if water is the solvent, no matter what mixture of substances make up the solutes. Thus, cytoplasm and water environments such as the oceans are all aqueous solutions.

In order to understand the many properties of water, and the importance of those properties to living organisms, we must first consider the structure of water molecules.

The covalent bonds between the oxygen atom and the two hydrogen atoms of a single water molecule are categorized as polar covalent bonds. You should remember from fundamental chemistry that covalent bonds form when two atoms share electrons. As electrons are negatively charged and the nucleus of an atom (because of the protons) is positively charged, any electrons that are shared equally create a bond and, because the charges cancel, this is called a non-polar covalent bond. The bond between two carbons is a good example of this type of bond. Polar covalent bonding results from an unequal sharing of electrons. In water, the single oxygen atom is bonded to two different hydrogen atoms. Each oxygen–hydrogen bond is a polar covalent bond, and results in a slight negative charge at the oxygen end of the molecule and a slight positive charge at the end with the two hydrogens. Because of the triangular shape of a water molecule, the two ends of each molecule have opposite charges, with

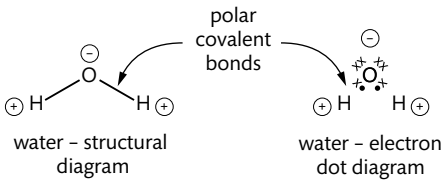


Figure 2.10 The shared electrons between oxygen and hydrogen are not shared equally, thus they are polar covalent bonds. This gives rise to the polarity of water.

the oxygen side being somewhat negative and the hydrogen side being somewhat positive. This is why water is a polar molecule: it has different charges at each end and so exhibits dipolarity. Because of this dipolarity, water molecules interact with each other and other molecules in very interesting ways. Many of these interactions are explained by the usually ephemeral (short-lived) attractions between either two water molecules or between water and another type of charged atom (or ion). These typically short-lived attractions are called hydrogen bonds and will be explained further in the following sections.

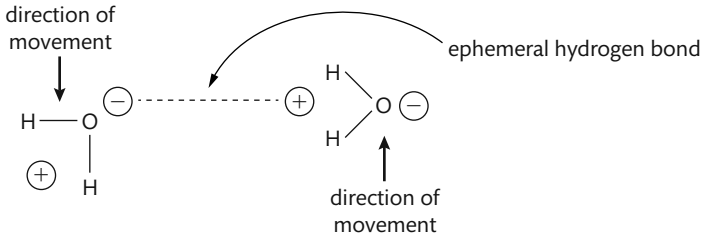


Figure 2.11 In liquid water, water molecules form 'split second' hydrogen bonds with other water molecules (dotted line), despite the fact that water continues to move in many different directions. These short-lived hydrogen bonds give rise to many of the interesting properties of water.

Cohesive properties

Water molecules are highly cohesive. Cohesion is when molecules of the same type are attracted to each other. As mentioned earlier, water molecules have a slightly positive end and a slightly negative end. Whenever two water molecules are near each other, the positive end of one attracts the negative end of another; this is hydrogen bonding. When water cools below its freezing point, the molecular motion has slowed to the point where these hydrogen bonds become locked into place and an ice crystal forms. Liquid water has molecules with a much faster molecular motion, and the water molecules are able to influence each other, but not to the point where molecules stop their motion. The ephemeral hydrogen bonding between liquid water molecules explains a variety of events, including:

- why water forms into droplets when it is spilt
- why water has a surface tension that allows some organisms to 'walk on water' (for some this is 'run on water')
- how water is able to move as a water 'column' in the vascular tissues of plants.

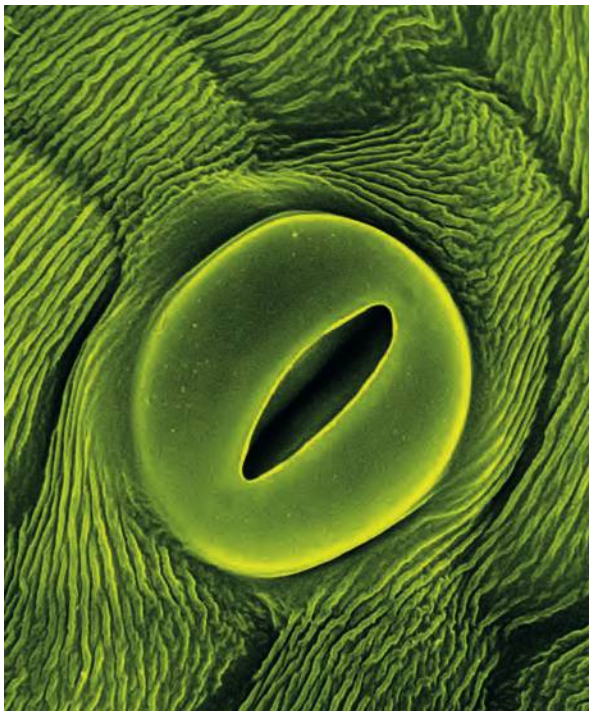
You can float a paper clip on water because of the surface tension of water. Make sure you maximize the surface area of the paper clip on the water if you try this.

A water strider making use of the high surface tension of water.

Adhesive properties

Water molecules are certainly not the only molecules in nature that exhibit polarity. Any attraction between two unlike molecules is called adhesion. Thus when water

molecules are attracted to cellulose molecules by hydrogen bonding, the attraction is an example of adhesion because the hydrogen bonding is between two different kinds of molecules. Where is this important in nature? One example is the column of water in plant vascular tissue, mentioned above. Cohesion and adhesion are both at work, because the water molecules exhibit cohesion to each other, and they also exhibit adhesion to the inside of the vascular tubes, which are partially composed of cellulose. When the column of water is 'pulled up', cohesion moves each molecule up a bit; when the column is not being 'pulled up', adhesion keeps the entire column from dropping down within the tube. The same phenomenon occurs when water is placed in a capillary tube; in fact, you can think of the vascular tissue in plants as being biological capillary tubes.



Water evaporates from leaves through small openings called stomata. As shown here, each stoma has two cells, called guard cells, that surround it. When the guard cells swell with water, the stoma appears between the cells, and water evaporates through the stoma. One benefit to the plant of this is the cooling effect that evaporation provides.

Thermal properties

Water has thermal properties that are important to living things. One of those thermal properties is high specific heat. In simple terms, this means that water can absorb or give off a great deal of heat without changing temperature very much. Think of a body of water on a very cold night: even though the air may be very cold, the body of water is relatively stable in temperature. All living things are composed of a great deal of water, and so you can think of your water content as a temperature stabilizer. Water also has a high heat of vaporization. This means that water absorbs a great deal of heat when it evaporates. Many organisms, including ourselves, use this as a cooling mechanism. Your internal body heat results in perspiration, and the perspiration then evaporates from your skin. Much of the heat that turned the water molecules from the liquid phase to the vapour phase came from your body, and thus sweating not only makes you feel cooler, it really does lower your temperature.

Solvent properties

Water is an excellent solvent of other polar molecules. You may remember from earlier science classes that like dissolves like. The vast majority of molecules typically found inside and outside most cells are also polar molecules. This includes carbohydrates, proteins, and nucleic acids (DNA and RNA). Most types of lipids are relatively non-polar and thus most organisms have special strategies to deal with the transport and biochemistry of lipids.

Because water is an excellent solvent for biochemically important molecules, it is also the medium in which most of the biochemistry of a cell occurs. A cell contains a wide variety of fluids, all of which are primarily water. We refer to such solutions as aqueous solutions. Table 2.3 shows some common aqueous solutions in which specific biochemical reactions take place.

The word 'stoma' comes from the Greek word meaning mouth or opening. In medicine, stoma is a surgically created opening in the body that replaces a normal opening.

Basilisk lizards may be as long as 0.8 m, but they can run across the surface of bodies of water. The relatively large surface area of their toes does not break through the surface tension of the water as long as they keep running.

Specific heat is the amount of heat per unit mass required to raise the temperature one degree Celsius.

Heat of vaporization is the amount of heat required to convert a unit mass of liquid into vapour with no increase in temperature.

Table 2.3 Common aqueous solutions

Aqueous solution	Location	Common reactions
Cytoplasm	Fluid inside cells but outside organelles	Glycolysis/protein synthesis reactions
Nucleoplasm	Fluid inside nuclear membranes	DNA replication/ transcription
Stroma	Fluid inside chloroplast membranes	Light-independent reactions of photosynthesis
Blood plasma	Fluid in arteries, veins, and capillaries	Loading and unloading of respiratory gases/clotting

Examples of water as a solvent in plants and animals

The properties of water make it an excellent medium for transport. Vascular tissue in plants carries water and a variety of dissolved substances. More specifically, xylem carries water and dissolved minerals up from the root system to the leaves of a plant. Phloem then transports dissolved sugars from the leaves to the stems, roots, and flowers of a plant.

Blood is the most common transport medium in animals, and is largely made up of water. The liquid portion of blood is called blood plasma. Some of the more common solutes in blood plasma are:

- glucose (blood sugar)
- amino acids
- fibrinogen (a protein involved in blood clotting)
- hydrogen carbonate ions (as a means of transporting carbon dioxide).

Water 'loving' or water 'fearing' substances

Molecules in living systems interact with water in a variety of ways. Remember that water is the solvent of life, and living cells typically have an aqueous environment both inside and outside their plasma membrane.

Molecules, such as water, that are polar substances are said to be hydrophilic, or water 'loving'. The majority of substances that are biochemically important are polar. Polar molecules easily dissolve in water, because a polar solvent will dissolve polar solutes. It is not difficult to recognize most of the molecules that are hydrophilic, as these molecules typically contain functional groups that result in the molecules being polar. Carbohydrates are a good example of polar molecules; their relative solubility in water is attributed to their multiple hydroxyl (alcohol) functional groups.

Molecules that are classified as non-polar are said to be hydrophobic, or water 'fearing'. Organic substances that are non-polar are typically composed of just carbons and hydrogens (hydrocarbons) or have large areas of the molecule where there are only carbons and hydrogens. Methane (CH_4) is an example of a hydrophobic molecule; it is composed of only one carbon and four hydrogens. Methane will not dissolve in water. Examples of biochemically important molecules that are predominately non-polar are the fatty acids found in triglyceride lipids and phospholipids. In addition to a carboxyl functional group at one end, a fatty acid consists of a long chain of carbons

TOK

Try doing a web search on the topic of 'memory of water'. Are any of the claims you find examples of pseudoscience rather than science?

with only hydrogens. The carboxyl group gives the fatty acid slight polarity at that end, but the chain of hydrocarbons is so long that the majority of the molecule is non-polar and thus hydrophobic.

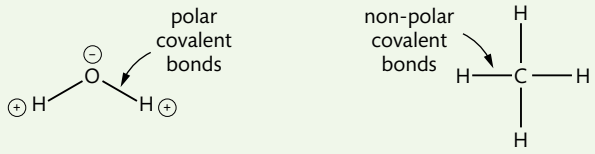
Protein molecules can be differentially polar depending on the arrangement of their amino acids. Some amino acids are relatively polar and some are non-polar. The location of each type of amino acid is important within the three-dimensional structure of the protein. Good examples are the proteins that attach into and extend out of a cell membrane. The amino acids making up the portion of the protein that attaches to (and extends down into) the membrane are hydrophobic and easily mix with the hydrophobic fatty acid 'tails' of the membrane phospholipid molecules. The portion of the protein that extends out of the membrane is predominately made up of hydrophilic amino acids that easily mix with the water environment either inside or outside the cell or organelle.

This photo shows what happens when a hydrophobic substance encounters water. The two types of molecules do not mix because they are not soluble.

NATURE OF SCIENCE

You probably already know the freezing point (0°C) and boiling point (100°C) of water. You may not already know the phase change temperatures for methane: the freezing point of methane is −183°C and the boiling point is −162°C.

It is interesting to think about why these two substances have such very different phase change temperatures. Consider the structure and polarity of these two molecules.



water H_2O methane CH_4

Figure 2.12 A comparison of water and methane.

The polar covalent bonds within water give rise to the polarity of the molecule. All of the covalent bonds within methane are non-polar and so methane is a non-polar substance. All molecules composed of just carbons and hydrogens (hydrocarbons) are non-polar.

When methane undergoes a phase change, because of its lack of polarity, there are no hydrogen bonds that influence the change of phase. You have probably realized that methane has a very low (cold) freezing point and also a very low boiling point. When methane changes from a liquid to a gas at −162°C there are no hydrogen bonds attracting the molecules to each other. Thus they 'escape' from each other with only a relatively small amount of molecular motion needed. That is not true for water molecules: each water molecule is constantly forming, breaking, and almost instantly reforming hydrogen bonds with other water molecules. When water changes from a liquid to a gas at 100°C, the high temperature is necessary to create the relatively high rate of molecular motion needed to enable the molecules to 'escape' from each other.

When methane changes from its liquid phase to its solid phase (at its freezing point, −183°C), the change in phase is explained by the fact that methane no longer has enough molecular motion to exist as a liquid. Water makes this phase change at a much higher temperature (0°C) because, when the molecular rate of motion becomes low enough, hydrogen bonding locks water molecules into stable geometric forms known as ice crystals.

We cannot actually see the hydrogen bonds. However, the theory that is used to explain hydrogen bonding is largely supported by many pieces of evidence, including those described above. Sometimes, in the nature of science, a theory helps explain a phenomenon and then multitudes of similar phenomena support the theory.

An artist's drawing of a cell membrane with proteins. The portions of the proteins found within the bilayer of phospholipids are composed of relatively non-polar amino acids, whereas those outside the bilayer are composed of many polar amino acids.

A typical person might be able to survive about 3 weeks without food. However, a typical person would only survive 1 week or less without any water intake.

How does solubility in water affect the mode of transport of molecules in organisms?

Water in living organisms acts as a mode of transport for the variety of molecules that must be moved about both within cells and between cells. Just think of the various water-based fluids you already know, such as cytoplasm, intercellular fluid, blood, and digestive juices. Because of their different polarities, each type of substance has a different solubility in whatever aqueous environment it is found in, including blood plasma. Table 2.4 summarizes the various relative polarities of a few selected molecules and shows whether or not an alternative mode of transport is needed as that substance circulates in the bloodstream.

Table 2.4 Polarity of different molecules

Substance	High or low relative solubility in water	Mode of transport in an aqueous environment (no special mode means the substance dissolves directly and easily into water)
Glucose	Polar molecule/high solubility	No special mode of transport needed/dissolves directly in aqueous plasma
Amino acids	Varying polarity but all are reasonably soluble	No special mode of transport needed/dissolve directly in aqueous plasma
Cholesterol	Largely non-polar/very low solubility	Transported by blood proteins that have polar amino acids on the outer portion to give water solubility, and non-polar amino acids internally to bind the non-polar cholesterol
Fats	Non-polar fatty acid components/very low solubility	Transported by blood proteins that have polar amino acids on the outer portion to give water solubility, and non-polar amino acids internally to bind the non-polar fatty acid molecules
Oxygen	Travels as diatomic O_2/ low solubility	Relatively low solubility in water is exacerbated by the relatively high temperature of warm-blooded animals (oxygen is less soluble in warm aqueous solutions)/haemoglobin is used to bind and transport oxygen molecules reversibly
Sodium chloride	Ionizes/high solubility	No special mode of transport needed/sodium chloride is an ionic compound, it ionizes into separately charged Na^+ and Cl^- ions in aqueous plasma

Exercises

4 Choose any specific aquatic or terrestrial animal and make a list of all the ways in which water is important to that animal.

5 How are the properties of water involved in any item of your list?

2.3 Carbohydrates and lipids

NATURE OF SCIENCE

Evaluating claims: health claims made about lipids in diets need to be assessed.

Understandings:

- Monosaccharide monomers are linked together by condensation reactions to form disaccharides and polysaccharide polymers.
- Fatty acids can be saturated, monounsaturated, or polyunsaturated.
- Unsaturated fatty acids can be *cis* or *trans* isomers.
- Triglycerides are formed by condensation from three fatty acids and one glycerol.

Applications and skills:

- Application: Structure and function of cellulose and starch in plants and glycogen in humans.
- Application: Scientific evidence for health risks of *trans* fats and saturated fatty acids.
- Application: Lipids are more suitable for long-term energy storage in humans than carbohydrates.
- Application: Evaluation of evidence and the methods used to obtain the evidence for health claims made about lipids.
- Skill: Use of molecular visualization software to compare cellulose, starch, and glycogen.
- Skill: Determination of body mass index by calculation or use of a nomogram.

Guidance

- *The structure of starch should include amylose and amylopectin.*
- *Named examples of fatty acids are not required.*
- *Sucrose, lactose, and maltose should be included as examples of disaccharides produced by combining monosaccharides.*

Humans and other animals have difficulty absorbing relatively large triglycerides and their digested form (fatty acids) from the intestine into the bloodstream. Chylomicrons are very small particles made up primarily of fat and some protein. Chylomicrons are produced in the alimentary canal and then transported into the bloodstream. They are used to transport fats to the liver and other tissues in the body. If your doctor orders a lipid blood test, chylomicrons are some of the low-density lipoproteins (LDL) that are measured. If their levels in the blood are elevated, they are referred to as the 'bad lipoproteins'.

Monosaccharides: the building blocks of disaccharides

Biochemically important molecules can be extremely large and complex but they are always made of smaller monomer (building block) molecules. The monomers of carbohydrates are the monosaccharides. At the beginning of this chapter you were introduced to hydrolysis reactions and an opposite set called condensation reactions. Condensation reactions are key to that part of your metabolism called anabolism, where larger molecules are synthesized from smaller monomer units. As the monomer units of carbohydrates are monosaccharides, we will start by looking at their structure. Monosaccharides can be classified according to how many carbon atoms they contain. The three most common monosaccharides are:

- trioses, containing 3 carbons and with the chemical formula $C_3H_6O_3$
- pentoses, containing 5 carbons and with the chemical formula $C_5H_{10}O_5$
- hexoses, containing 6 carbons and with the chemical formula $C_6H_{12}O_6$.

You may have noticed a common pattern in the formulas of these three simple sugars: monosaccharides typically fit the formula $C_nH_{2n}O_n$, where n equals the number of carbon atoms.

Some textbooks will refer to condensation reactions as dehydration synthesis reactions. The names condensation and dehydration synthesis are both good reminders that water is always one of the products of these reactions.

Let's look at a detailed example of a condensation reaction occurring between two monosaccharides. The example in Figure 2.13 shows the formation of the disaccharide sucrose from the reaction between the two monosaccharides glucose and fructose.

In similar reactions, other disaccharides are formed by different monosaccharides undergoing a condensation reaction. Figure 2.14 shows the condensation reaction that forms the disaccharide maltose from two alpha-D-glucose molecules. In a very similar way, the disaccharide lactose is formed by the condensation reaction between alpha-D-glucose and the monosaccharide galactose.

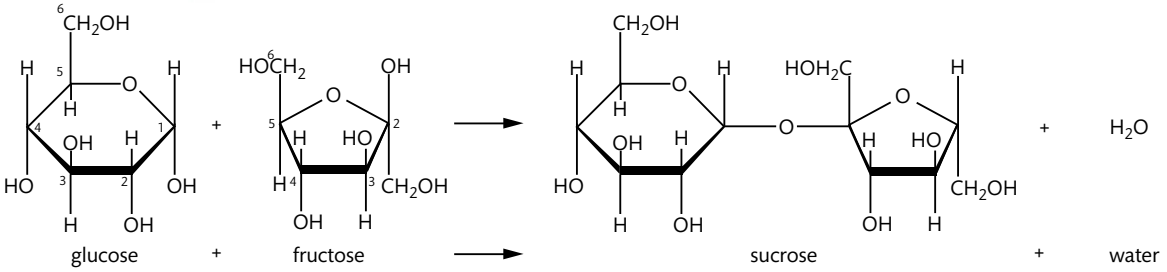
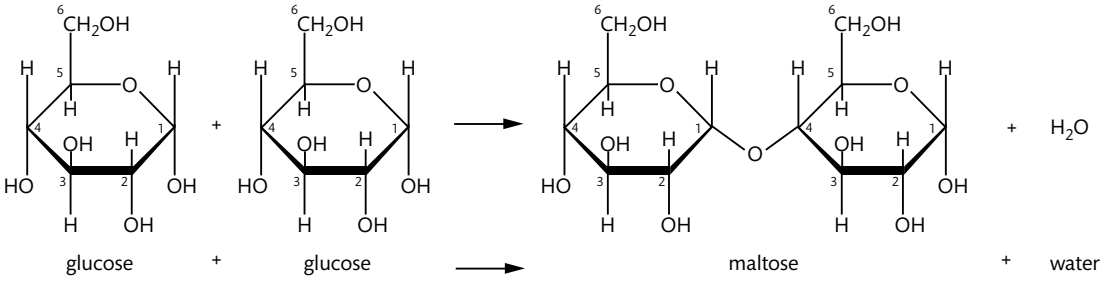


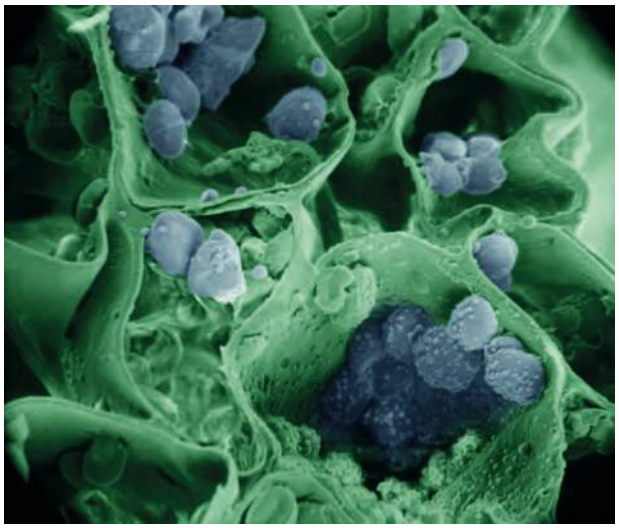
Figure 2.13 The condensation reaction between glucose and fructose to form the disaccharide sucrose and a water molecule. Each corner of the sugar rings has an 'unshown' carbon atom. Each carbon atom is numbered in the reactants. Glucose and fructose are isomers of each other because they have the same chemical formula, $C_6H_{12}O_6$.

Figure 2.14 A condensation reaction showing the formation of the disaccharide maltose. Notice that water is always a product of a condensation reaction and that one of the two monosaccharides 'donates' a hydroxide ion (^-OH) and the other monosaccharide 'donates' a hydrogen ion (H^+), which combine to form the water molecule. The bond that is freed up is used to form the covalent bond between the two monosaccharides. All condensation reactions occur in a very similar way.



Monosaccharides: the building blocks of polysaccharides

Condensation reactions can be used to synthesize even larger molecules by accomplishing the same or a similar reaction on more than one area of a monomer such as a monosaccharide. Repeatedly bonding glucose monosaccharides produces a variety of very large molecules or polymers. Some examples are cellulose, starch, and glycogen; Table 2.5 summarizes their functions.



Scanning electron micrograph (SEM) of sliced open plant cells. The plant cell walls composed largely of cellulose are clearly visible, and in the interior of the cells are chloroplasts, which produce and store carbohydrates such as starch.

Table 2.5 The functions of major polysaccharides

Polysaccharide	Summary of functions
Cellulose	Major component of plant cell walls, helps give rigidity/support to plant parts such as roots, stems, and leaves
Starch	Organic products of photosynthesis are stored in plants as starch, typically as starch granules in chloroplasts or in plant storage areas such as roots or root structures
Glycogen	Animals store excess glucose in this form. Glycogen is stored in the liver and in muscle tissue

At the end of this section, use the hotlinks to view and manipulate three-dimensional models of cellulose, starch, and glycogen. When viewing these structures online, take note of the following.

- Cellulose, starch, and glycogen are all polysaccharides of the same monomer unit, glucose.
- The bonding mentioned with each molecule, such as 1,4 linkages, refers to the carbon numbers of the glucose molecules that create the covalent bond.
- Starch has two subcomponents, amylopectin and amylose.
- Amylose is the only one of the three glucose polysaccharides that is a linear molecule with no side branching.
- All three polysaccharides can be composed of many thousands of glucose monomers.

Fatty acids

Although they have similarities in their molecular structure, not all fatty acids are identical. All fatty acids have a carboxyl group (–COOH) at one end and a methyl group (CH_3–) at the other end. In between is a chain of hydrocarbons (hydrogen atoms and carbon atoms) that is usually between 11 and 23 carbons long (12–24 carbons when counting the carbon of the methyl group as well).

Saturated fatty acids

In Figure 2.15, the yellow zone on the left is the carboxyl group, the white zone in the middle is the hydrocarbon chain (shown much shorter than any fatty acid in the human body), and the green zone on the right is where the methyl group is located.

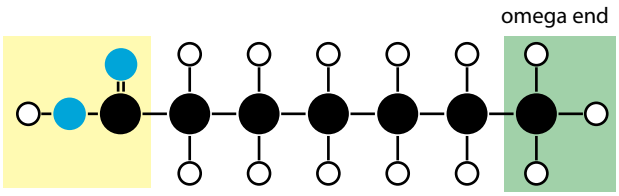


Figure 2.15 The three sections found in all fatty acids: the carboxyl group at one end, the long hydrocarbon chain in the middle, and the methyl group at the other end. The end with the methyl group is also called the omega end.

Saturated fatty acids are called that because the carbons are carrying as many hydrogen atoms as they can, in other words they are saturated with hydrogen atoms. These molecules are typically found in animal products such as butter, bacon, and the fat in red meat. These fats are generally solid at room temperature. Because the carbons are carrying as many hydrogen atoms as possible, saturated fatty acids have no double bonds between the carbon atoms. The shape of the molecule is straight: there are no kinks or bends along the chain.

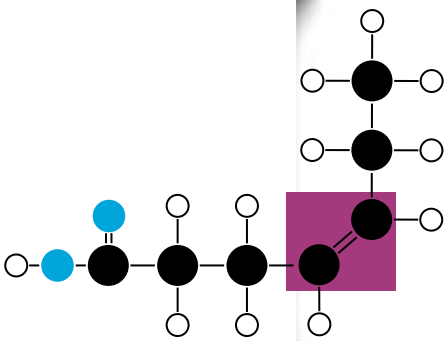


Figure 2.16 The highlighted zone in the middle of the fatty acid shows that it has a single double bond in the hydrocarbon chain. This creates a bend or kink in the shape of the molecule. Note: Fatty acids typically have more carbons than the one shown for this illustration.

Monounsaturated fatty acids

If one double bond exists in the chain of hydrocarbons, the fatty acid is not saturated any longer: it has two empty spaces where hydrogen atoms could be. This type of unsaturated fatty acid is referred to as monounsaturated.

In Figure 2.16, the double bond between two carbons in the hydrocarbon chain is highlighted. Notice how the absence of two consecutive hydrogen atoms on the same side of the carbon atom chain causes the molecule to bend at the zone where the double bond is.

Polyunsaturated fatty acids

Polyunsaturated fatty acids have at least two double bonds in the carbon chain. They typically come from plants (olive oil is an example). These fatty acids are called polyunsaturated because two or more carbons are not carrying the maximum number of hydrogen atoms (another way of saying this is that two or more carbons are double bonded to each other). Lipids that contain polyunsaturated fatty acids tend to be liquids at room temperature.

Imagine a hydrocarbon chain several times longer than any shown in the figures so far, with several more double bonds. The molecule may have so many bends/kinks that it starts to curve over onto itself or twist around itself. This frequently happens with polyunsaturated fatty acids.

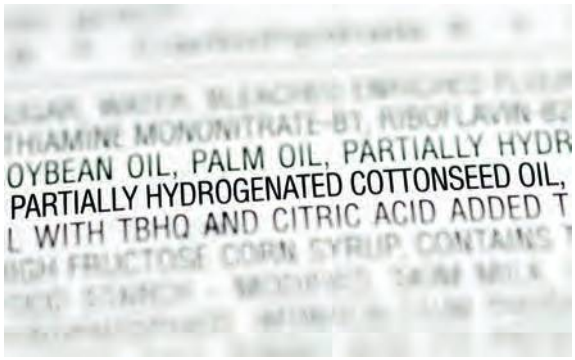
The calorie count based on lipid content is important for many people in order to maintain a healthy weight, but the type of lipids found in foods that gives those calories should be important to everyone.

Hydrogenation: *cis* and *trans* fatty acids

In many heavily processed foods, polyunsaturated fats are often hydrogenated or partially hydrogenated as part of the processing. This means the double bonds (and hence the kinks) are eliminated (or partly eliminated) by adding hydrogen atoms. Hydrogenation straightens out the natural bent shape of unsaturated fatty acids. Naturally curved fatty acids are called *cis* fatty acids, and the hydrogenated,

straightened ones are called *trans* fatty acids. The vast majority of *trans* fatty acids are the result of chemical transformations in food-processing factories. They are usually only partially hydrogenated and thus still contain one or more double bonds.

One category of *cis* fatty acids is called omega-3. The name comes from the fact that the first carbon double bond to be found in this molecule is at the third carbon atom counting backwards from the omega end (see Figure 12.17). Fish are a good source of omega-3 fats.



Part of the ingredients list of a bought cake. 'Partially hydrogenated' means that this is a product that contains *trans* fats.

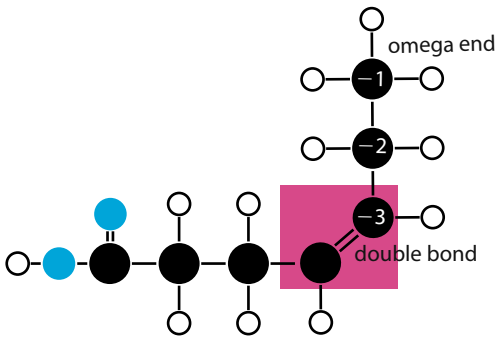


Figure 2.17 This sketch shows how the name omega-3 is derived for some fatty acids. Starting at the omega carbon, count the carbons until you reach the first double bond.

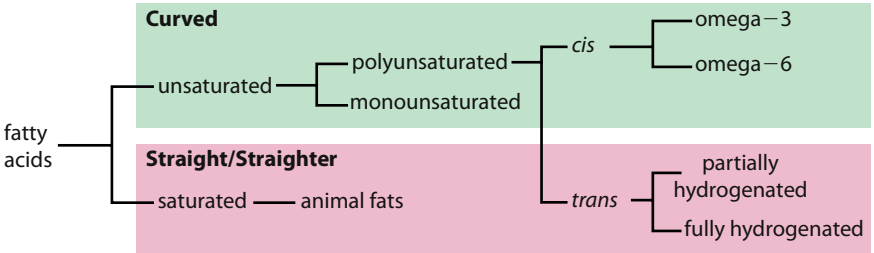


Figure 2.18 Summary of fatty acid types.

Condensation reactions result in the formation of triglyceride lipids

The component molecules of triglyceride lipids (fats in animal cells and oils in plant cells) are glycerol and three fatty acids. The identity and thus characteristics of the three fatty acids in each triglyceride will determine the overall characteristics of the fat or oil. Triglycerides vary greatly from each other, including their relative healthiness in our diet. Figure 2.19 shows a representation of the condensation reaction that creates the covalent bonds between the glycerol portion and the three fatty acids of a triglyceride lipid. Notice that, as in all condensation reactions, a water molecule is created from each of the three reactions.

Condensation and hydrolysis reactions in biochemistry are so common that you will encounter information concerning those two types of reactions throughout your study of biology. Take the time to learn the basics of these two reaction types.

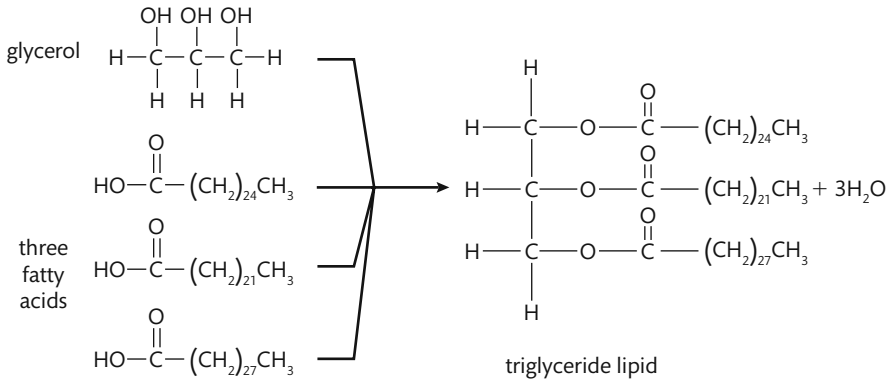


Figure 2.19 Condensation reaction showing the four reactants necessary to form a triglyceride lipid. Notice that there are four products: the three water molecules as well as the triglyceride.

NATURE OF SCIENCE

Now that you are familiar with the terminology and basic chemical structure of lipids and fatty acids, you are ready to read and evaluate some information concerning various types of lipids in your foods. Try to evaluate the information given by researching the following.

Use a search engine to research consumer information reported by food companies concerning lipids. Try:

- one or more of your favourite fast food restaurants (or at least some you know) and couple the restaurant name with 'nutrition information'
- the company and snack name of one or more of your favourite snacks plus 'nutrition information'
- other searches that you can think of that may or may not give you reasonably reliable information.

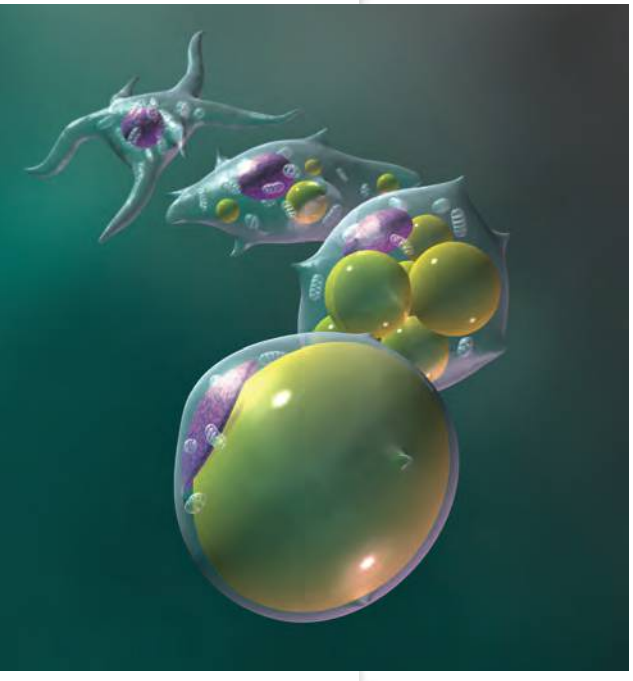
Diets characteristic of people in various areas of the world appear to have a huge influence on health and longevity.

Energy storage solutions in humans

Humans and many other organisms have developed chemical strategies to store molecules in reserve to use for ATP production during the process of cell respiration. These include:

- storing glucose as the polysaccharide glycogen in liver and muscle tissues
- storing triglyceride lipids within adipose (fat) cells.

This drawing shows a fat cell (adipocyte) becoming larger as lipids are stored in it.



Triglyceride lipids, when needed, can be hydrolysed into two carbon segments that can enter into cell respiration at a chemical sequence point that is very efficient for the production of ATP. Thus lipids have about twice the energy content per gramme compared with other molecules, such as carbohydrates and proteins, that are also used for cell respiration.

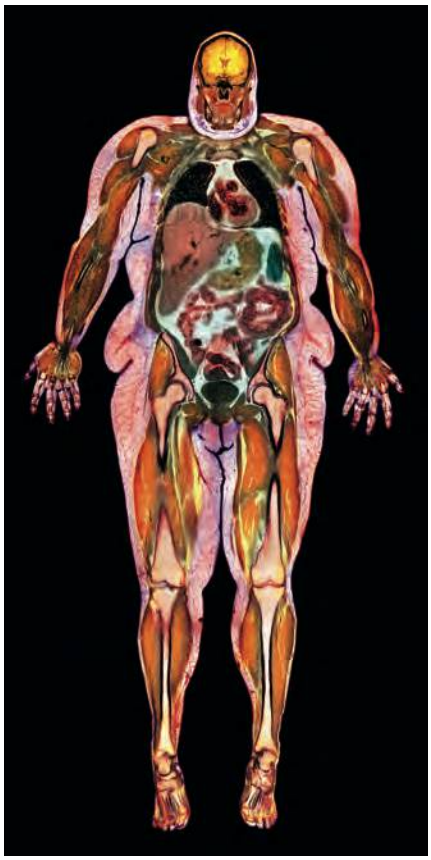
Lipids have another advantage as a long-term energy storage molecule: they are insoluble in water (such as in the aqueous environments of cytoplasm, intercellular fluid, and blood plasma), and so they do not upset the osmotic balance of solutions. If humans were to store large concentrations of glucose in certain cells of the body for long-term energy storage, those cells would swell to ridiculous proportions because the glucose would attract water into the cells due to the surrounding hypotonic fluids.

Calculating the body mass index

The use of an indexed value known as the body mass index (BMI) as an indicator of healthy weight has recently become popular. The BMI is a number that reflects both the weight and the height of a person. The idea is that people who are taller should weigh more. There are three ways that you can determine your BMI:

- using a formula, based on either metric or imperial measurements of weight and height
- using a graph known as a nomogram to read the BMI value from a central intersection point between weight and height measurements
- using an online calculator that outputs the BMI after the height and weight measurements have been input.

Each of the methods used to determine the BMI must be correlated with information concerning the BMI that shows whether a value reflects someone being underweight, normal in weight, overweight, or obese. Such charts often come with a caution that states children and pregnant women should not use them. Table 2.6 shows the data provided by the Centers for Disease Control and Prevention (CDC).



Colorized magnetic resonance image (MRI) of a woman with a very high BMI. Among a myriad of other possible problems, the extra body mass present in obese patients puts a strain on their heart and lungs.

Some, but not all, countries make a concerted effort to inform their citizens of the health risks and benefits of certain foods/diets. This is why good scientific research on the consequences and benefits of certain food types is essential.

Table 2.6 Interpreting BMI values

BMI	Description category
Below 18.5	Underweight
18.5–24.9	Normal weight
25.0–29.9	Overweight
30.0 and above	Obese

Here are the two formulas for calculating BMI:

- formula 1, metric units, BMI = weight (kg)/[height (m) × height (m)]
- formula 2, imperial units, BMI = weight (lb)/[height (in) × height (in)] × 703

Example 1 (metric): for someone who is 1.70 m and weighs 58 kg, his or her BMI = 58/(1.7 × 1.7) = 20.1. Therefore this person is categorized as having a normal weight.

Example 2 (imperial): for someone who is 5′10″ (5′10″ = 70″) and weighs 235 lb, his or her BMI = 235/(70 × 70) × 703 = 33.7 . This person is categorized as obese.

CHALLENGE YOURSELF

3 Calculate the BMI of a person who is 1.64 m tall and weighs 79 kg. Using Table 2.6, which category would be used to describe him or her?

4 Calculate your own BMI after measuring your height and current weight.

At the end of this section, use the hotlinks to go to a website that includes a nomogram and online calculator for determining BMI.

5 Use the online calculator to confirm your own BMI calculation.

6 Use the nomogram to confirm your own BMI calculation.

To learn more about the three-dimensional models of cellulose, starch, and glycogen, and calculating BMI, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.3.

Exercises

6 Write the word equation for the condensation reactions that would produce a triglyceride lipid from its four molecular subcomponents.

7 Rank these fatty acids types from the least to the most healthy: saturated fatty acid; unsaturated fatty acid; *trans* fatty acid.

8 Why is BMI a better reflection of a person's health compared with body mass alone?

NATURE OF SCIENCE

Looking for patterns, trends, and discrepancies: most but not all organisms assemble proteins from the same amino acids.

2.4 Proteins

Understandings:

- Amino acids are linked together by condensation to form polypeptides.
- There are 20 different amino acids in polypeptides synthesized on ribosomes.
- Amino acids can be linked together in any sequence, giving a huge range of possible polypeptides.
- The amino acid sequence of polypeptides is coded for by genes.
- A protein may consist of a single polypeptide or more than one polypeptide linked together.
- The amino acid sequence determines the three-dimensional conformation of a protein.
- Living organisms synthesize many different proteins with a wide range of functions.
- Every individual has a unique proteome.

Applications and skills:

- Application: Rubisco, insulin, immunoglobulins, rhodopsin, collagen, and spider silk as examples of the range of protein functions.
- Application: Denaturation of proteins by heat or by deviation of pH from the optimum.
- Skill: Drawing molecular diagrams to show the formation of a peptide bond.

Guidance

- *The detailed structure of the six proteins selected to illustrate the functions of proteins is not needed.*
- *Egg white or albumin solutions can be used in denaturation experiments.*
- *Students should know that most organisms use the same 20 amino acids in the same genetic code, although there are some exceptions. Specific examples could be used for illustration.*

Formation of polypeptides

Cells use the naturally occurring 20 amino acids to synthesize polypeptides. They do this under the control of DNA, each polypeptide being created under the control of a specific area of a specific DNA molecule called a gene. In a multicellular organism, every cell of that organism has the same set of chromosomes and thus the same DNA. Each cell that has differentiated to have a specific function in a specific tissue of the body only uses the genes that are necessary for that cell type. Some of those genes are

almost universal, such as the genes that code for proteins involved in common cell functions. A good example of this would be the protein components that make up ribosomes, as all cells need ribosomes. In addition, each specific cell type then uses the genes that help accomplish the specific activities necessary for that cell type. A cell of the human pancreas would 'turn on' the gene for synthesis of the peptide hormone insulin, whereas most cells would not activate that gene even though the gene is present in all human cells. The total number of (possibly) active genes in any living organism is difficult to determine with accuracy. A current estimate for human beings is somewhere between 20 000 and 25 000 genes in each of our cells. This is nothing to brag about though, as a high gene count falls somewhere between grape plants (which have about 30 000 genes) and chickens (which have about 17 000 genes). This shows why it would be a mistake to correlate the number of genes with organism complexity. Table 2.7 shows a selection of organisms and their approximate gene count.

Table 2.7 Selected organisms and their approximate number of genes

Common name of the organism	Approximate number of genes in the organism's genome
Yeast (single-celled fungi)	6 000
Drosophila (fruit fly)	14 000
Rice plant	51 000
Laboratory mouse	30 000
Domestic dog	19 000
Humans	20–25 000

No matter how many genes an organism has within its genome, all genes are the genetic code for the possible polypeptides found within that organism, and all polypeptides are synthesized from the same monomers, specifically amino acids. Although there are a few exceptions to this, virtually all organisms use the same genetic code, and they use the same 20 amino acids to construct their polypeptides.

Each of the 20 amino acids differs from the others in one bonding location around the central carbon atom; that difference in structure is called the R or variable group of the amino acid (Figure 2.20). You do not need to memorize the R-groups but you do need to memorize the general structure that applies to all amino acids.

When amino acids are in an aqueous solution, such as cytoplasm or blood plasma, the amine and carboxyl functional groups ionize, as shown in Figure 2.20. This ionization does not alter the covalent bonding pattern but it does make the functional groups look a little different as each carboxyl group has 'lost' a hydrogen ion and each amine group has gained a hydrogen ion.

When polypeptides are synthesized at ribosomes under the control of genes, the reaction that is occurring is a condensation reaction. The sequence of the amino acids is determined precisely by the DNA, but the condensation reactions are virtually identical.

Polypeptides are highly variable

The condensation reactions described above do not occur between any two amino acids randomly. The order of the amino acids is always determined by triplets of nucleotides

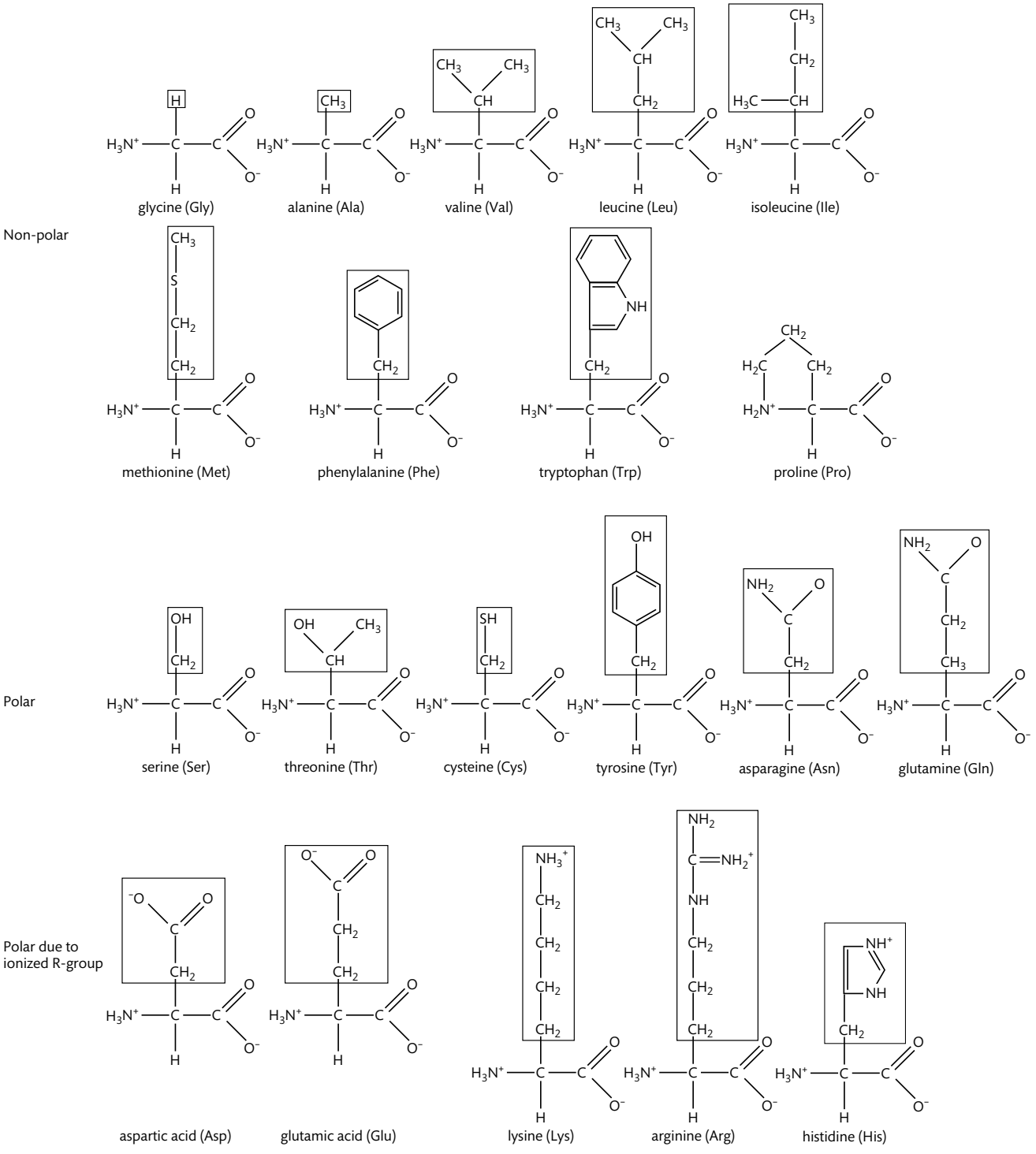


Figure 2.20 A chart showing the structures of the 20 amino acids. The boxed areas shown are the R-groups of the amino acids. Note how each amino acid is identical except for the variable R-group.

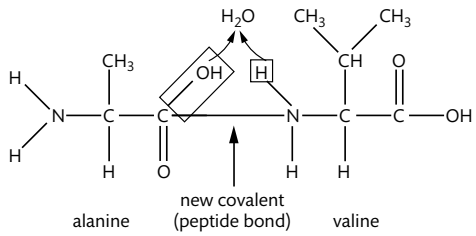
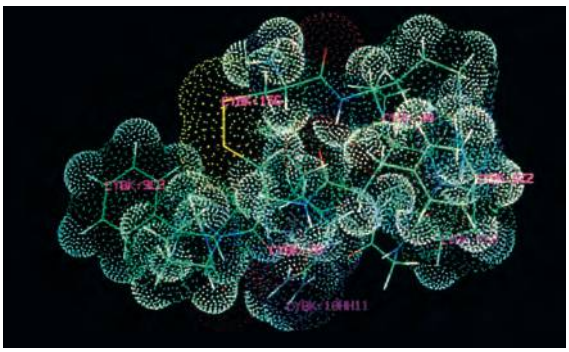


Figure 2.21 Condensation reaction between the amino acids alanine and valine. Note that for simplicity the amine and carboxyl groups are being shown in a non-ionized form. This reaction looks the same for any two amino acids, as the only change would be to the R (variable) groups.

along nucleic acid molecules (DNA and RNA), and is directed by a ribosome. As there are 20 amino acids, there is a large choice for the sequence of the amino acids as well as the total number of amino acids to use within a polypeptide. Each polypeptide that has been selected for a specific purpose has not only its own amino acid sequence, but also its own three-dimensional shape; that shape has a dominant influence on the function of the polypeptide. Even a change in a single amino acid in the overall sequence of a polypeptide can have drastic effects on its function.



Computer graphic representation of the structure of bradykinin, a polypeptide that is active in human metabolism. Despite the apparent complexity, this is a relatively short peptide consisting of only nine amino acids.

Levels of polypeptide and protein structure

Proteins serve a tremendous variety of functions in cells and organisms; Table 2.8 shows you just a few examples.

Table 2.8 Some examples of proteins and their functions

Rubisco	The short-hand name for the enzyme that catalyses the first reaction of the carbon-fixing reactions of photosynthesis
Insulin	A protein hormone produced by the pancreas that results in a decrease of blood sugar levels and an increase of sugar inside body cells
Immunoglobulin	Another name for an antibody that recognizes an antigen(s) as part of the immune response
Rhodopsin	A pigment found in the retina of the eye that is particularly useful in low light conditions
Collagen	The main protein component of connective tissue, which is abundant in skin, tendons, and ligaments
Spider silk	A fibrous protein spun by spiders for making webs, drop lines, nest building, and other uses

Given the myriad of functions of proteins, they have to be capable of assuming many forms and structures. The function of any particular protein is closely related to its structure. There are four levels of organization to protein structure: primary, secondary, tertiary, and quaternary.

- Primary protein structure: the sequence of amino acids within the protein; this sequence determines the three-dimensional shape, as shown below.
- Secondary protein structure: repetitive shapes of either a helix (a spiral staircase shape) or a pleated sheet (a sheet with corrugated folds), e.g. spider silk.
- Tertiary structure: a shape often described as globular, e.g. enzymes.
- Quaternary: two or more polypeptides combined together to make a single functional protein, e.g. haemoglobin.

CHALLENGE YOURSELF

7 Use the amino acid structures in Figure 2.20 and draw the following short peptide consisting of five amino acids. The *n*-terminal end begins with an amine group and the *c*-terminal end finishes with a carboxyl group.
n-terminal end [Valine – Glycine – Serine – Threonine – Alanine] *c*-terminal end

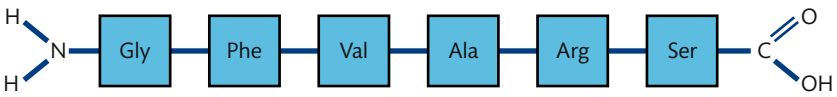


Figure 2.22 Simplistic example of a polypeptide's primary structure.

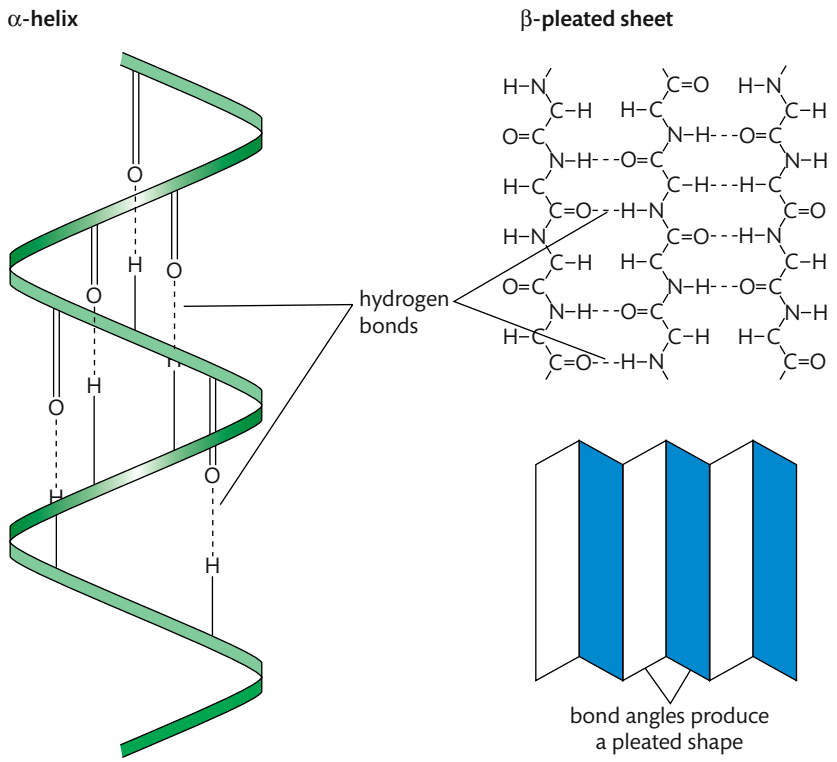


Figure 2.23 The two geometric patterns of protein secondary structures. The variable or R-groups are not shown in secondary structures as they are not involved in creating the molecular shape.

When trying to identify individual amino acids within a large, complex polypeptide, try to identify the peptide bonds between each of the covalently bonded amino acids. That bond will always be a nitrogen atom bonded to a carbon atom, with that carbon atom also doubled bonded to an oxygen.

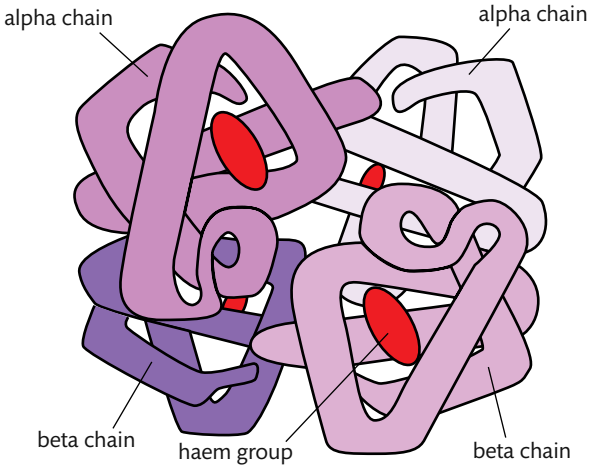


Figure 2.24 Molecular model of the protein structure of haemoglobin. Each haemoglobin molecule is considered to be a single protein. Each contains four polypeptide chains held together in a quaternary structure. Some of the same types of bonds important for creating the tertiary structure also help to hold quaternary structure proteins together.

Some proteins are more than one polypeptide

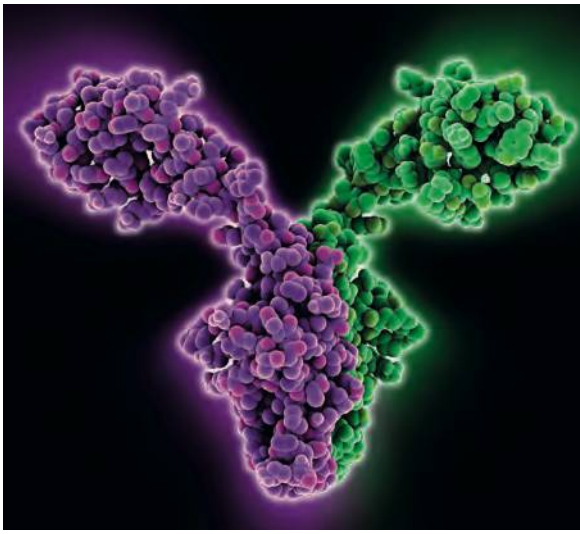
Frequently, the terms polypeptide and protein are used interchangeably. In fact, based on the biochemistry of proteins, the two terms do have a slightly different meaning. A protein is an organic substance consisting of covalently bonded amino acids, and it is ready to carry out its function. If the protein is an enzyme, it is ready to catalyse

a reaction. If the protein is an antibody (immunoglobulin), it is ready to bond to an antigen as part of an immune response. The point is, a protein is able to carry out its intended function. That may or may not be true for a polypeptide.

A polypeptide is a single amino acid chain with its own primary structure. It has a single *c*-terminal end and a single *n*-terminal end. If the single polypeptide is able to carry out its function as it is, then that polypeptide is considered to be a protein.

Some polypeptides cannot serve a biochemical function until they combine with one or more other polypeptide(s). If you recall, this is what is called a quaternary structure. When two or more polypeptides bond together and then are ready to accomplish their function, together they are considered to be a single protein.

This is a protein found in the ribosomes of some bacteria. The computer model of the protein clearly shows that the protein is composed of two polypeptides. Each of the polypeptides would require a different gene within the bacteria's genome to code for its synthesis.



Your unique proteome

Over the last few decades we have come to know that each individual organism of a species is genetically different from all other organisms. This is especially true for organisms that reproduce by sexual reproduction. The specific DNA sequence that is unique to one individual is called a genome. As DNA is the genetic code for proteins, this means that each individual has a unique set of proteins that he or she is capable of synthesizing. Thus each individual is said to have a unique proteome as well as a unique genome.

Proteins can be denatured by heat and alteration of the pH environment

The intra-molecular bonds of proteins that hold together their secondary, tertiary, and quaternary structures are susceptible to alterations in normal temperature and pH; the intra-molecular bonds can be disrupted. When a protein takes on a three-dimensional shape, it does this because of the interactions of the amino acids with each other.

When protein molecules are placed into a temperature environment that is higher than their physiological optimum, the increased molecular motion puts a great deal of stress on many of the relatively weak intra-molecular bonds. This can result in the primary structure remaining intact (the sequence of amino acids connected by peptide bonds) but the hydrogen bonds often cannot stay in place under the stress caused by the increased molecular motion. The result is that the protein loses its normal three-dimensional shape and function. A protein's function is directly dependent on its shape; in most instances, as long as the covalent bonds (like peptide bonds) remain intact, the protein will return to its normal shape and function if it is returned to its normal temperature.

A similar phenomenon occurs when a protein is placed in a pH environment that is not close to its optimum pH. A protein will lose its normal three-dimensional shape, and thus lose its functionality, in these circumstances. When a fluid environment such as cytoplasm, blood plasma, etc., is flooded with either H^+ ions (an acid) or ^-OH ions (a base), the extra charges can prevent normal hydrogen bonding. Thus the protein will not take on its 'normal' shape and will not function normally.

For centuries people have been selectively breeding both crops and animals to increase their food value. Recently, some companies have begun genetically modifying foods using biotechnology. The jury is still out regarding whether this approach will ultimately be both beneficial and safe.

Technically, a proteome is the collection of proteins found within a particular cell type at a specified time under a specific set of environmental circumstances. Cells in multicellular organisms differentiate and thus do not produce the same proteins even though they contain the same genome.

Some living organisms have evolved proteins and other molecules that remain stable and functional at very high temperatures. This is a hot spring called Morning Glory in Yellowstone National Park, USA. The brilliant colours you see in the water are primarily the result of the growth of cyanobacteria that can live in water temperatures as high as 165°C.

Exercises

9 Study the amino acid chart (Figure 2.20) and find the amino acids that meet the following criteria.

- **(a)** The single amino acid whose non-R-group shape is slightly different compared with all the others.
- **(b)** The two amino acids that contain sulfur atoms.
- **(c)** The five amino acids that contain either a carboxyl or an amine group as part of their R-group.

10 How many peptide bonds would be found in a polypeptide that contains 76 amino acids?

11 Considering only the usual 20 naturally occurring amino acids, how many combinations of amino acids would be possible if four amino acids were to bond together in a random order?

NATURE OF SCIENCE

Most, but not all, organisms assemble proteins from the same 20 amino acids. Virtually every reference concerning amino acids will tell you that there are 20 amino acids in nature. It is true that the universal genetic code (universal indicating that it is used in the vast majority of organisms on Earth) only encodes 20. But in nature there are frequently exceptions, and that includes things that are called 'universal'. If you include all known living organisms then there are 22 amino acids that are used to create polypeptides. In addition to the 20 amino acids whose structures are given in Figure 2.20, there are two additional amino acids called selenocysteine and pyrrolysine.

2.5 Enzymes

Understandings:

- Enzymes have an active site to which specific substrates bind.
- Enzyme catalysis involves molecular motion and the collision of substrates with the active site.
- Temperature, pH, and substrate concentration affect the rate of activity of enzymes.
- Enzymes can be denatured.
- Immobilized enzymes are widely used in industry.

Applications and skills:

- Application: Methods of production of lactose-free milk and its advantages.
- Skill: Design of experiments to test the effect of temperature, pH, and substrate concentration on the activity of enzymes.
- Skill: Experimental investigation of a factor affecting enzyme activity.

Guidance

- *Lactase can be immobilized in alginate beads, and experiments can then be carried out in which the lactose in milk is hydrolysed.*
- *Students should be able to sketch graphs to show the expected effects of temperature, pH, and substrate concentration on the activity of enzymes. They should be able to explain the patterns or trends in these graphs.*

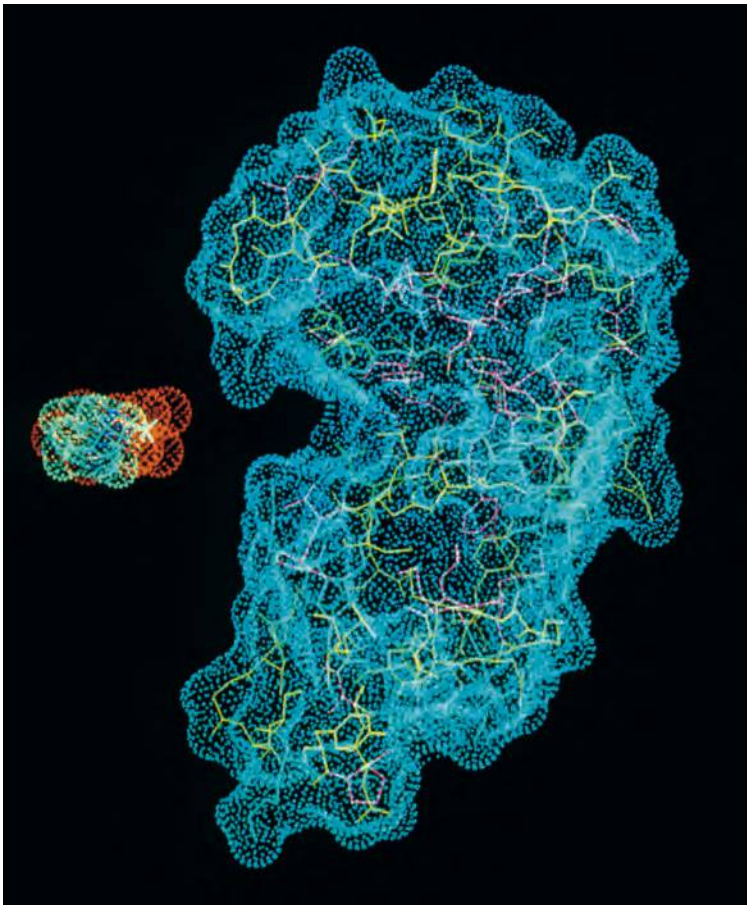
NATURE OF SCIENCE

Experimental design: accurate, quantitative measurements in enzyme experiments require replicates to ensure reliability.

Enzymes are organic molecules that act as catalysts

Enzymes are proteins. Thus enzymes are long chains of amino acids that have taken on a very specific three-dimensional shape. Think of a flexible metal wire that can be

bent many times into what is called a globular shape. This shape is complex and at first glance appears to be random, but in enzymes (and other globular proteins) the complex shape is not random: it is very specific. Somewhere in the three-dimensional shape of the enzyme is an area that is designed to match a specific molecule known as that enzyme's substrate. This area of the enzyme is called the active site. The active site of an enzyme matches the substrate in a similar way to a glove fitting a hand. In this analogy, the glove represents the active site and the hand represents the substrate.



This computer graphic shows an enzyme (the larger molecule on the right) and its substrate. Notice the active site on the left-hand side of the enzyme.

Another analogy that is very commonly used for enzyme–substrate activity is a lock and key. In this analogy, the lock represents the enzyme's active site and the key represents the substrate. Because the three-dimensional shape of the internal portion of the lock is complex and specific, only one key will fit. The same principle is generally true for enzymes and their substrates: they are specific for each other.

It is not enough for an enzyme's substrate(s) to just enter an active site. The substrate(s) must enter with a minimum rate of motion that will provide the energy necessary for the reaction to occur. Enzymes do not provide this energy, they simply lower the energy minimum that is required. The energy being referred to is called the activation energy of the reaction. Thus enzymes lower the activation energy of reactions. Enzymes are not considered to be reactants and are not used up in the reaction. An enzyme can function as a catalyst many, many times. In addition, an enzyme cannot force a reaction to occur that would not otherwise happen without the enzyme; however, the reaction may be much more likely to occur with an enzyme because the input of energy (activation energy) required will be lower with the enzyme present.

Factors affecting enzyme-catalysed reactions

When you are considering the various environmental factors that affect enzyme-catalysed reactions, you must first remember that all chemical reactions are fundamentally molecules colliding. If the molecules that are colliding do so at a high enough rate of speed and the molecules have the capability of reacting with each other, then there is a chance that a reaction will occur. Enzymes cannot change those fundamentals.

Effect of temperature

Imagine an enzyme and its substrate floating freely in a fluid environment. Both the enzyme and substrate are in motion and the rate of that motion is dependent on the temperature of the fluid. Fluids with higher temperatures will have faster moving molecules (more kinetic energy). Reactions are dependent on molecular collisions and, as a general rule, the faster molecules are moving, the more often they collide, and with greater energy. Reactions with or without enzymes will increase their reaction rate as the temperature (and thus molecular motion) increases. Reactions that use enzymes do have an upper limit, however (see Figure 2.25). That limit is based on the temperature at which the enzyme (as a protein) begins to lose its three-dimensional shape because the intra-molecular bonds are being stressed and broken. When an enzyme loses its shape, including the shape of the active site, it is said to be denatured. Denaturation is frequently temporary, as in many instances the intra-molecular bonds will re-establish when the temperature returns to a suitable level.

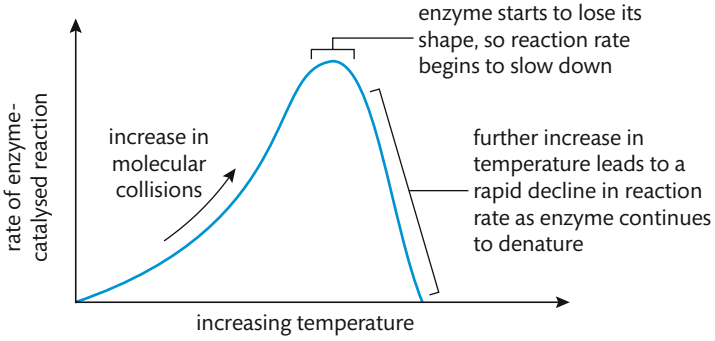


Figure 2.25 The effect of increasing temperature on the rate of an enzyme-catalysed reaction.

Whether or not an enzyme is permanently destroyed by denaturation is largely dependent on whether covalent bonds (such as peptide bonds) have broken. DNA determines the order of amino acids, and they have no way of reassembling properly if they become detached from each other.

Effect of pH

The active site of an enzyme typically includes many amino acids of that protein. Some amino acids have areas that are charged either positively or negatively. The negative and positive areas of a substrate must match the opposite charge when the substrate is in the active site of an enzyme, in order for the enzyme to have catalytic action. When a solution has become too acidic, the relatively large number of hydrogen ions (H^+) can bond with the negative charges of the enzyme or substrate, and prevent proper charge matching between the two. A similar scenario occurs when a solution has become too basic: the relatively large number of hydroxide ions (^-OH) can bond with the positive charges of the substrate or enzyme, and once again prevent proper charge matching between the two. Either of these scenarios will result in an enzyme becoming less efficient, and in extreme situations becoming completely inactive. One further possibility is that the numerous extra positive and negative charges of acidic and basic solutions can result in the enzyme losing its shape and thus becoming denatured.

There is no one pH that is best for all enzymes (see Figure 2.26). Many of the enzymes active in the human body are most active when in an environment that is near neutral. There are exceptions to this, however; for example, pepsin is an enzyme that is active in the stomach. The environment of the stomach is highly acidic and pepsin is most active in an acidic pH.

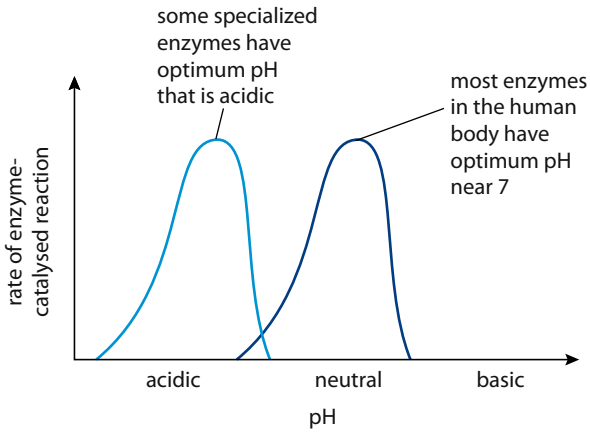


Figure 2.26 The effect of pH on the rate of an enzyme-catalysed reaction. This illustrates that there is no single pH that is best for all enzymes.

Effect of substrate concentration

If there is a constant amount of enzyme, as the concentration of a substrate increases, the rate of reaction will increase as well (see Figure 2.28). This is explained by the idea of increased molecular collisions. If you have more reactant molecules, there are more to collide. There is a limit to this, however, because enzymes have a maximum rate at which they can work. If every enzyme molecule is working as fast as possible, adding more substrate to the solution will not increase the reaction rate further (see Figure 2.28).

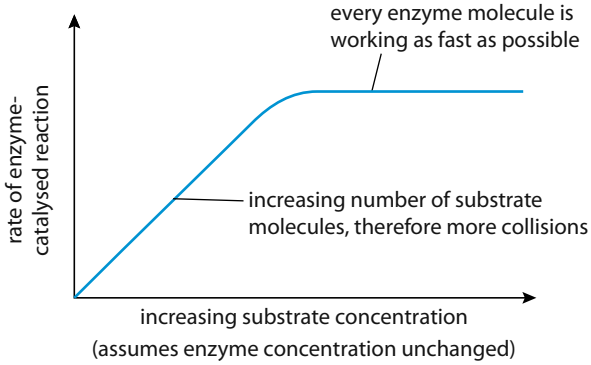


Figure 2.28 The effect of increasing the substrate concentration on the rate of an enzyme-catalysed reaction.

Use of immobilized enzymes in industry

Cells are not the only 'factories' that make good use of enzymes. In the last 50 years, many industrial applications have been developed that make use of these catalytic proteins. However, there are major problems that have to be overcome. For example, if you want to catalyse one particular reaction, you need a pure enzyme, not a mixture as found in cells. Extracting or producing pure enzymes in the large quantities needed for industrial use is expensive. Because of their cost, enzymes in industry need to be reused repeatedly. The problem is that it is difficult to remove enzymes from liquid products in solutions so that the enzymes can be used further. One answer to this

	pH
strongly acidic	1
	2
	3
	4
weakly acidic	5
	6
neutral	7
weakly alkaline	8
	9
strongly alkaline	10
	11
	12
	13
	14

Figure 2.27 The pH scale. Most fluids within the human body are close to neutral. The pH of blood plasma is typically 7.4, making it very slightly alkaline.

The pH scale is a logarithmic scale. This means that each whole number on the pH scale represents an increase or decrease by a power of 10. Thus a solution with a pH of 4 has 10 times more relative hydrogen ions compared with a solution with a pH of 5. That same solution with a pH of 4 has 100 times more relative hydrogen ions compared with a solution with a pH of 6.

problem is to invent ways to trap the enzymes in place and prevent them from getting washed out with the products. Researchers found that an enzyme could be held in place in tiny pores on beads of a substance called calcium alginate. Those enzymes trapped in the pores are said to be immobilized. As long as the alginate beads are recovered in the industrial process, the enzymes are also recovered and can be reused.

Use of immobilized lactase to produce lactose-free milk

The majority of humans are born with the ability to digest lactose, one of the most common sugars found in milk. The reason for this is that we are born with the ability to produce the enzyme lactase in our digestive tract. Lactase is the enzyme that digests the disaccharide lactose into two monosaccharides (glucose and galactose); the monosaccharides are much more readily absorbed into the bloodstream. Most people lose the ability to produce lactase as they get older, and by adulthood no longer produce any significant amount of lactase. These people are said to be lactose intolerant. Normal milk and milk products enter their digestive tract and are not digested; instead the normal bacterial colonies in their intestines feed directly on the lactose. In effect, these bacterial colonies are being overfed. This leads to symptoms such as cramping, excessive gas, and diarrhoea.

It has been found that there is an extremely high incidence of lactose intolerance in some ethnic groups and a relatively low incidence in others. This is a good example of natural variation in a population.

In order to avoid these unpleasant symptoms, people who are lactose intolerant can eat milk and milk products that have been treated with lactase before consumption. With this treatment, the nutrients in the milk are not affected but the disaccharide lactose has been pre-digested, so a lactose-intolerant person is able to absorb the monosaccharide sugars.

There are more people with lactose intolerance than there are people who do not have the condition. In genetics, lactose intolerance is called the wild-type (the most common phenotype in a natural population).

One of the ways to pre-digest milk products on a large industrial scale is to use the method described above. Specifically, lactase enzyme molecules are trapped in the small pores of alginate beads and then milk and milk products are exposed to these beads for enough time for pre-digestion to occur.

Investigation of factors affecting enzyme activity

Safety alerts: Eye protection and lab aprons should be worn for all stages of these experiments.

Enzymes are protein catalysts. The catalytic ability of an enzyme can be optimized in certain pH and temperature environments, as well as by increasing the substrate concentration available to the enzyme. Because enzymes are proteins, they are subject to the same denaturing factors that affect all other proteins, including pH environments that are far from their optimum, and temperature environments that put stress on their internal bonds that help shape the molecule.

Note: This lab is designed for a class to be divided into three groups, each assigned one of the following questions.

1. What is the effect of altering the pH environment on the activity of the enzyme lactase? Hypothesis for question 1: the optimum pH environment for lactase will be slightly acidic (pH 6.0–6.5).
2. What is the effect of altering the temperature environment on the activity of the enzyme lactase? Hypothesis for question 2: the optimum temperature environment for lactase will be 25°C.
3. What is the effect of altering the concentration of substrate (lactose) on the activity of the enzyme lactase? Hypothesis for question 3: the optimum substrate concentration for lactase will be a ratio of 20 parts lactose by mass to 1 part lactase by mass.

- The following locally available reagents will need to be purchased: lactose powder (available from food shops), lactase powder or tablets, and glucose test strips (available from pharmacies). An alternative to using glucose test strips is to use Benedict's reagent, following standard protocols. An alternative for lactose powder is milk; use powdered milk if you want to compare the ratio of lactose mass to lactase mass, as in question 3.

- In addition, pH strips or another means of measuring the pH of solutions will be needed for the pH group, as well as buffered solutions for the desired pH. Bulb thermometers will be needed for the temperature group, and a mass scale for the substrate concentration group.
- Standard glassware and supplies, such as stirring rods, spatulas, test tubes, beakers, etc., will also be needed, based on your chosen techniques for carrying out the tests.

Safety alerts: Eye protection and lab aprons should be worn for all stages of these experiments

- To make the enzyme solution (lactase), crush and add one lactase tablet to 200 ml water. Stir well until completely dissolved.
- To make the substrate solution (lactose), starting with powdered milk, follow the instructions given with the powder, and then decant the volumes needed.
- To carry out a negative control test (one that is designed to purposely give negative results), test the lactose solution using either a glucose test strip or Benedict's reagent (to show the absence of glucose).
- To carry out a positive control test (one that is designed to purposely give positive results), in a test tube add 2 ml of liquid milk and 1 ml of enzyme solution. Immediately mix well and start a timer. Test the solution for the presence of glucose after each 1-minute time period until the test is positive for glucose. Record the time necessary to achieve this positive result.

Each group will need to use the above standard procedures to design and carry out their own investigation by altering the solution pH, solution temperature, or the ratio of the mass of substrate to mass of enzyme (this mass ratio investigation should be based on the mass of the substrate and enzyme when in powder/tablet form). The dependent variable in each investigation will be the time necessary to achieve a positive glucose test.

Commercially available lactase has been formulated to still be active in the stomach and so is not sensitive to alterations in various acidic pH environments. Thus this investigation should attempt to start at a slightly acidic pH and have various increments to (safe) alkaline solutions.

Commercially available lactase is also quite temperature tolerant and will not completely denature until boiled for about 30 minutes.

Exercises

12 Briefly explain why enzymes and substrates are specific for each other.

13 Why are enzymes considered to be catalysts of reactions?

14 How much more acidic is a solution of pH 3 compared with a solution of pH 6?

To learn more about enzymes, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2.5

2.6 Structure of DNA and RNA

Understandings:

- The nucleic acids DNA and RNA are polymers of nucleotides.
- DNA differs from RNA in the number of strands present, the base composition, and the type of pentose.
- DNA is a double helix made of two antiparallel strands linked by hydrogen bonding between complementary base pairs.

Applications and skills:

- Application: Crick and Watson's elucidation of the structure of DNA using model making.
- Skill: Drawing simple diagrams of the structure of single nucleotides of DNA and RNA, using circles, pentagons, and rectangles to represent phosphates, pentoses, and bases.

Guidance

- *In diagrams of DNA structure, the helical shape does not need to be shown, but the two strands should be shown antiparallel. Adenine should be shown paired with thymine, and guanine with cytosine, but the relative lengths of the purine and pyrimidine bases do not need to be recalled, nor the numbers of hydrogen bonds between the base pairs.*

NATURE OF SCIENCE

Using models as representations of the real world: Crick and Watson used model making to discover the structure of DNA.

For many years most scientists all over the world believed it was protein, not DNA, that contained our genetic information. Research conducted in the first few decades of the 20th century demonstrated that DNA contains our genetic blueprint.

Nucleotides are the building blocks of nucleic acids

As you learned earlier in this chapter, nucleic acids are one of the major carbon-based groups. There are three major examples of nucleic acids in nature. They are adenosine triphosphate (ATP), deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). ATP functions as an energy storage compound. Other nucleic acids function as coenzymes. In this section we will focus on DNA and RNA. DNA and RNA are involved with the genetic aspects of the cell.

Both DNA and RNA are polymers of nucleotides. Individual nucleotides are referred to as monomers and always consist of three major parts: one phosphate group, one 5-carbon monosaccharide, and a single nitrogenous base. Chemical bonds occur at specific locations in order to produce a functional unit. Look at Figure 2.29.

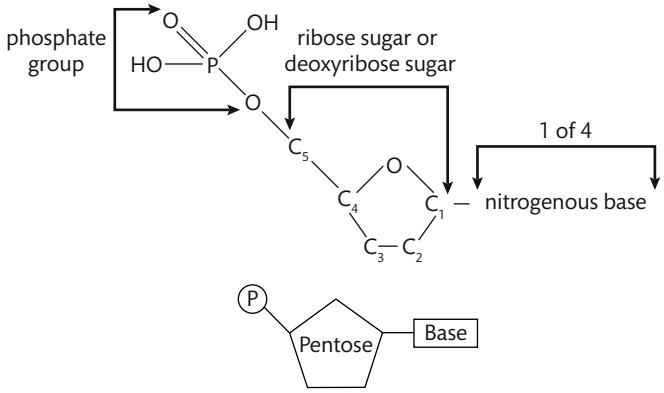


Figure 2.29 The first diagram represents the structure of a nucleotide showing bond locations. The second diagram represents the structure of a general nucleotide using the symbols suggested by the IB.

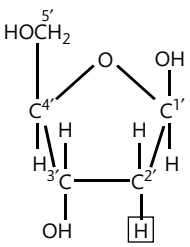
It is important to note that in the diagram circles are used to represent phosphates, pentagons are used to represent 5-carbon sugars (also called pentoses), and rectangles are used to represent nitrogenous bases. All IB drawings involving nucleotides should use these symbols.

All the bonds within the nucleotide involve the sharing of electrons, and are therefore referred to as covalent bonds. The phosphate group is the same in DNA and RNA. However, there are five possible nitrogenous bases, which are shown in Table 2.9.

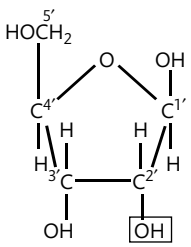
Table 2.9 The five nitrogenous bases

RNA nitrogenous bases	DNA nitrogenous bases
Adenine (A)	Adenine (A)
Uracil (U)	Thymine (T)
Cytosine (C)	Cytosine (C)
Guanine (G)	Guanine (G)

Figure 2.30 Nucleotide sugars.



Deoxyribose



Ribose

The base uracil only occurs in RNA, not DNA, and the base thymine only occurs in DNA, not RNA. When drawing nucleotides, it is common practice to put the capitalized first letter of the base inside the rectangle.

The sugar differs in the nucleotides of DNA and RNA. DNA nucleotides contain the pentose known as deoxyribose and RNA nucleotides contain ribose. In Figure 2.30, you can see that they are very similar molecules.

Monomers into polymers

Monomers (single nucleotides) in both DNA and RNA may bond together to produce long chains or polymers. An example of such a chain is shown in Figure 2.31.

In Figure 2.31 each adjoining nucleotide has been drawn in a different colour to emphasize the nucleotide structure. Notice that the chain has an alternating pentose–phosphate backbone, with the nitrogenous bases extending outward. The importance of the order of these nitrogenous bases will be discussed later in conjunction with the genetic code. The nucleotides attach to one another to form a chain as a result of condensation reactions forming connecting covalent bonds.

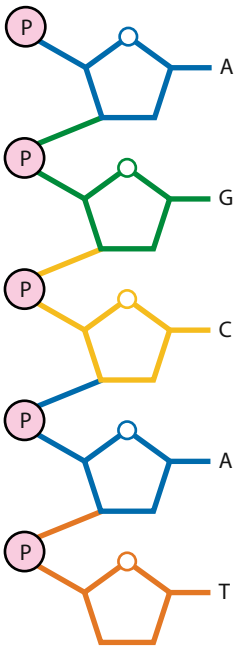


Figure 2.31 Five nucleotides bonded to form a very small section of a strand of DNA or RNA.

Single strand or double strand

RNA is composed of a single chain or strand of nucleotides, while DNA consists of two separate chains or strands of nucleotides connected to one another by weak hydrogen bonds. The strands of both DNA and RNA may involve very large numbers of nucleotides. For the two strands of DNA, imagine a double-stranded DNA molecule as a ladder (see Figure 2.32). The two sides of the ladder are made up of the phosphate and deoxyribose sugars. The rungs of the ladder (what you step on) are made up of the nitrogenous bases. Because the ladder has two sides, there are two bases making up each rung. The two bases making up one rung are said to be complementary to each other. The complementary base pairs are adenine (A)–thymine (T) and cytosine (C)–guanine (G).

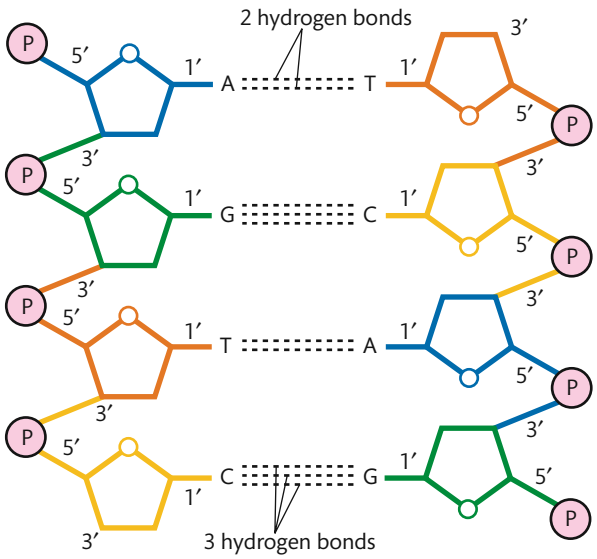


Figure 2.32 A small section of a double-stranded DNA molecule showing hydrogen bonds between complementary nitrogenous bases. The two single strands that make up the double-stranded molecule run in opposite directions to each other. The term that describes this is 'antiparallel'. Thus we say that the two strands of the double helix are antiparallel and complementary to each other.

CHALLENGE YOURSELF

8 Use the symbols mentioned on page 86 to represent all the possible nucleotides of DNA. Once you have done that for DNA, do the same for RNA.

CHALLENGE YOURSELF

9 Examine the first diagram in Figure 2.29 representing the general structure of a nucleotide. Notice that the carbons of the pentose are numbered. These numbers are always placed in this way for both ribose and deoxyribose. Now look at Figure 2.31, in which five nucleotides are connected together. Answer the following.

(a) In the polymer, which numbered carbons are always attached to the phosphate group?

(b) In a monomer, what number carbon is always attached to the phosphate group?

(c) Which carbon is always attached to the nitrogenous base?

Even though the first accurate model of DNA was produced by James Watson (American) and Francis Crick (British) in 1953, many other scientists from around the world contributed pieces of information that were instrumental in developing the final model. Erwin Chargaff (Austrian) had determined that the numbers of adenine and thymine bases were equal, as were the numbers of cytosine and guanine bases. Rosalind Franklin (British) and Maurice Wilkins (born in New Zealand) had calculated the distance between the various molecules in DNA by X-ray crystallography.

We can now use all of this information to construct a simple, yet accurate, drawing of DNA.

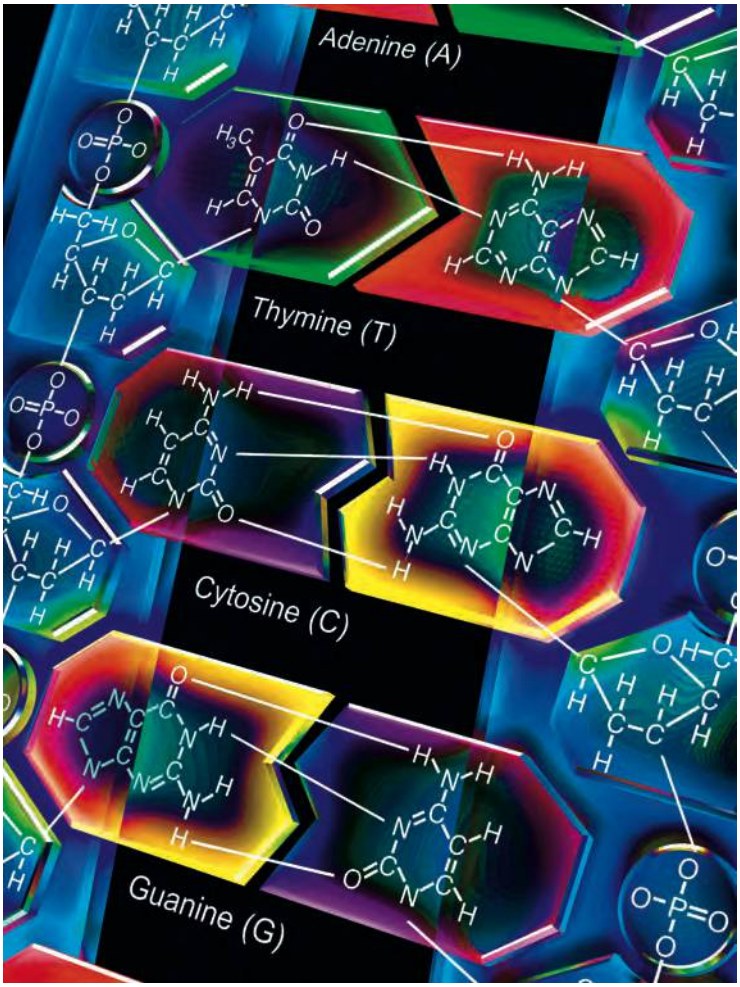


Figure 2.33 This artwork shows complementary base pairs and hydrogen bonding in DNA. Note that thymine and cytosine are much smaller molecular structures than adenine and guanine.

In Figure 2.32, it is essential to note that one strand of DNA has the 5-carbon, often referred to as the 5-prime (5′) carbon, unattached and on top. At the bottom of that same strand notice that the 3- or 3-prime (3′) carbon is unattached. If you look at the opposite strand of deoxyribose and phosphates, you will notice it is the opposite: the 3′ carbon is at the top and the 5′ carbon is at the bottom. These two strands are therefore said to be antiparallel to one another. Electrical charges related to the molecules of the two strands cause a characteristic twisting action of the DNA ladder to produce the double helix shape that Watson and Crick described in the model they proposed in the early 1950s.

Even though some information was exchanged, the development of the first accurate model of DNA was highly competitive. Several groups in different parts of the world were trying to make sense of shared knowledge to produce an appropriate model. Some scientists did not share their research or findings. How is this 'anti-scientific'? Discuss what can be done to increase the sharing of personal knowledge in scientific research.

NATURE OF SCIENCE

Francis Crick and James Watson used models to arrive at the structure of DNA. They used data from many different sources to construct this model successfully. They did not have the ability to observe the molecule directly, which made the model necessary. The model they produced was an actual physical model, using wires and symbols representing atoms. Today, many models are produced using computer-based mathematical models. Regardless of how a model is produced, it is always subject to modification as more experiments are conducted and more data are collected.

CHALLENGE YOURSELF

10 In order to better understand the basic structures of RNA and DNA, it is useful to compare and contrast their characteristics. They are actually quite similar. When comparing two compounds, using a *t*-chart or a table is recommended. *t*-charts may take many forms, but all allow a direct comparison between related items or materials. In this case, complete the table below, which allows a comparison of the two compounds.

Feature	RNA	DNA
Number of strands		
Bases present		
Pentose present		
Name of monomers		

Table 2.10

Exercises

15 Why do researchers often give DNA information as the sequence of nitrogenous bases without indicating the presence of the phosphate group and sugar component of each nucleotide?

16 Starting with a blank piece of paper, practise drawing a ladder diagram of DNA in which the nitrogenous base sequence of one strand is C, T, G, G, A, T. Be sure to include a representation of the phosphate groups and deoxyribose sugar in each nucleotide.

To learn more about DNA structure, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.6.

2.7 DNA replication, transcription, and translation

Understandings:

- The replication of DNA is semi-conservative and depends on complementary base pairing.
- Helicase unwinds the double helix and separates the two strands by breaking hydrogen bonds.
- DNA polymerase links nucleotides together to form a new strand, using the pre-existing strand as a template.
- Transcription is the synthesis of mRNA copied from the DNA base sequences by RNA polymerase.
- Translation is the synthesis of polypeptides on ribosomes.
- The amino acid sequence of polypeptides is determined by mRNA according to the genetic code.
- Codons of three bases on mRNA correspond to one amino acid in a polypeptide.
- Translation depends on complementary base pairing between codons on mRNA and anticodons on tRNA.

Application and skills:

- Application: Use of *Taq* DNA polymerase to produce multiple copies of DNA rapidly by the polymerase chain reaction (PCR).
- Application: Production of human insulin in bacteria as an example of the universality of the genetic code allowing gene transfer between species.
- Skill: Use a table of the genetic code to deduce which codon(s) corresponds to which amino acid.
- Skill: Analysis of Meselson and Stahl's results to obtain support for the theory of semi-conservative replication of DNA.
- Skill: Use a table of mRNA codons and their corresponding amino acids to deduce the sequence of amino acids coded by a short mRNA strand of known base sequence.
- Skill: Deducing the DNA base sequence for the mRNA strand.

Guidance

- *The different types of DNA polymerase do not need to be distinguished.*

NATURE OF SCIENCE

Obtaining evidence for scientific theories: Meselson and Stahl obtained evidence for the semi-conservative replication of DNA.

Helicase can catalyse the unzipping of DNA at a rate measured in hundreds of base pairs per second.

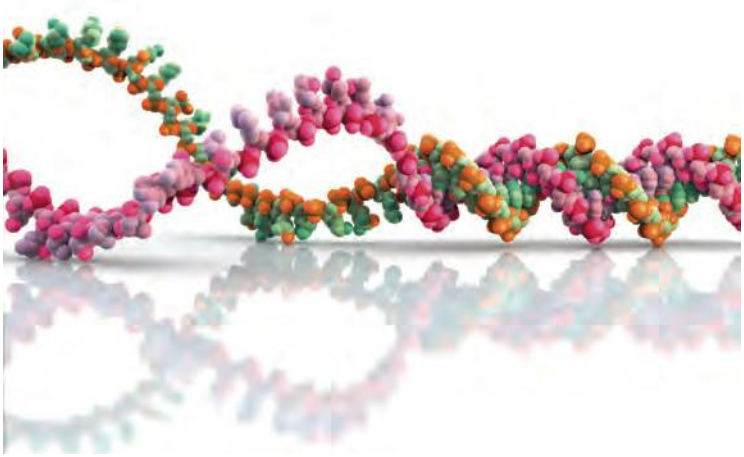
DNA replication involves 'unzipping'

Cells must prepare for a cell division by doubling the DNA content of the cell in a process called DNA replication. This process doubles the quantity of DNA and also ensures that there is an exact copy of each DNA molecule. In the nucleus of cells are two types of molecules that are particularly important for the process of DNA replication; they are:

- enzymes needed for replication, which include helicase and a group of enzymes collectively called DNA polymerase
- free nucleotides, which are nucleotides that are not yet bonded and are found floating freely in the nucleoplasm, some contain adenine, some thymine, some cytosine, and some guanine.

One of the early events of DNA replication is the separation of the double helix into two single strands. You should remember that the double helix is held together by the hydrogen bonds between complementary base pairs (adenine and thymine, cytosine and guanine). The enzyme that initiates this separation into two single strands is called helicase. Helicase begins at a point in or at the end of a DNA molecule, and moves one complementary base pair at a time, breaking the hydrogen bonds so the double-stranded DNA molecule becomes two separate strands.

Helicase (currently at about the half-way point in this image of a DNA double helix being unzipped) would have started on the left and be moving towards the right.



The unpaired nucleotides on each of these single strands can now be used as a template to help create two double-stranded DNA molecules identical to the original. Some people use the analogy of a zipper for this process. When you pull on a zipper, helicase is like the slide mechanism. The separation of the two sides of the DNA molecule is like the two opened sides of a zipper. See Figure 2.34.

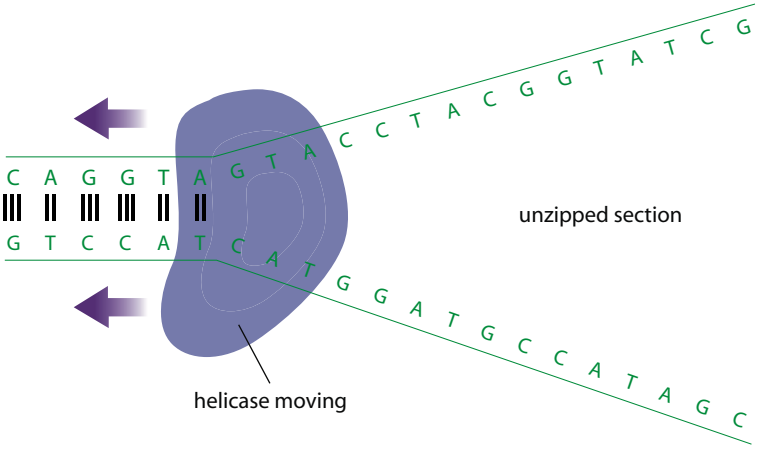


Figure 2.34 The first step of DNA replication is helicase unzipping the double-stranded DNA molecule, forming a section with two single strands.

Formation of two complementary strands

As shown in Figure 2.34, once DNA has become unzipped, the nitrogenous bases on each of the single strands are unpaired. In the environment of the nucleoplasm, there

are free-floating nucleotides. These nucleotides are available to form complementary pairs with the single-stranded nucleotides of the unzipped molecule. This does not happen in a random fashion. A free nucleotide locates on one opened strand at one end, and then a second nucleotide can join the first. This requires these two nucleotides to become covalently bonded together, because they are the beginning of a new strand. The formation of a covalent bond between two adjoining nucleotides is catalysed by one of the DNA polymerase enzymes that are important in this process.

A third nucleotide then joins the first two, and the process continues in a repetitive way for many nucleotides. The other unzipped strand also acts as a template for the formation of another new strand. This strand forms in a similar fashion, but in the opposite direction to the first strand. In Figure 2.35, notice that one strand is replicating in the same direction as helicase is moving and the other strand is replicating in the opposite direction.

The significance of DNA replication is that it ensures that two identical copies of DNA are produced from one original. The diagram illustrates a very small section of DNA replicating.

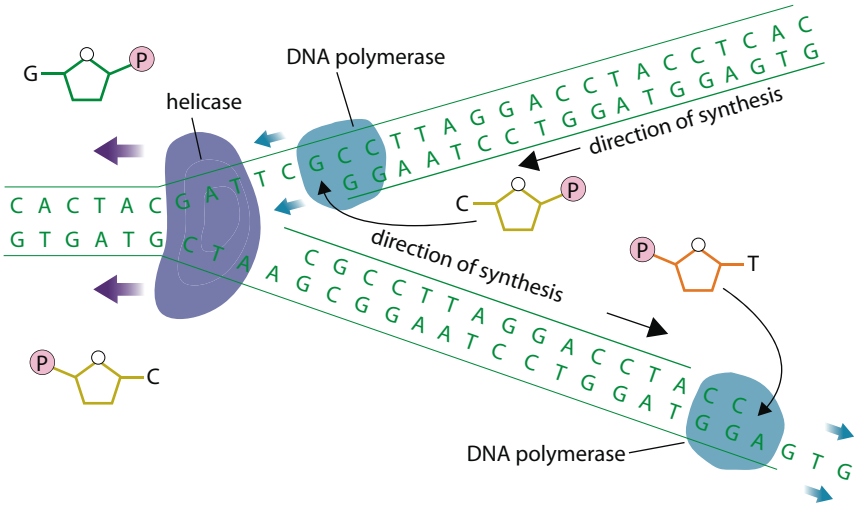
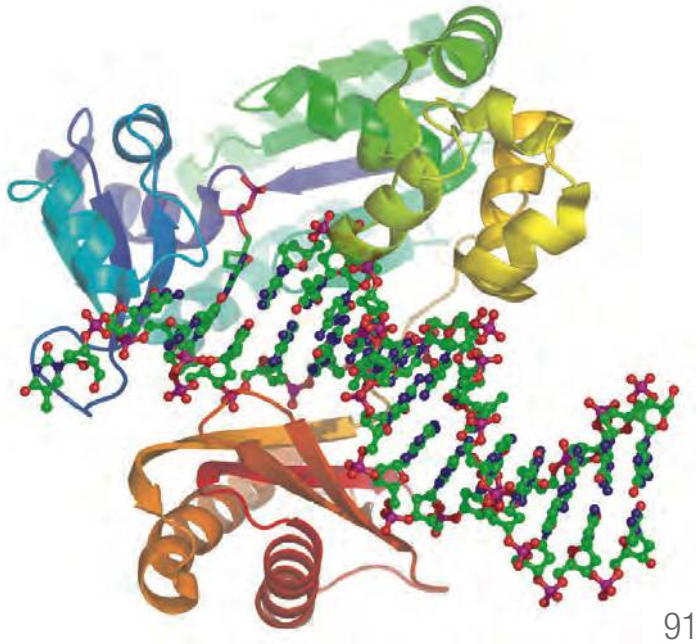


Figure 2.35 DNA replication

Figure 2.36 A small section of DNA (shown in the centre of this artwork) is seen in a DNA polymerase enzyme.



Notice that in the area where replication has already taken place, the two strands are absolutely identical to each other. This is because the original double-stranded molecule had complementary pairs of nucleotides and it was the complementary nucleotides that used the unzipped single-stranded areas as templates.

This also means that no DNA molecule is ever completely new. After replication, every DNA molecule consists of a strand that is 'old' paired with a strand that is 'new'. DNA replication is described as a semi-conservative process because half of a pre-existing DNA molecule is always conserved (saved).

CHALLENGE YOURSELF

11 The experimental work that determined that DNA replication was semi-conservative is often called 'the most beautiful experiment in biology'. This experiment was carried out by Matthew Meselson and Frank Stahl, with their results published in 1958. An overview of the experiment and the data obtained follows.

- Two separate cultures of *Escherichia coli* bacteria were grown with the presence of either a 'heavy' isotope of nitrogen, ^{15}N, or an ordinary 'light' isotope of nitrogen, ^{14}N.
- After many generations, the DNA in each bacteria culture contained either the heavy form or the light form of nitrogen. The nitrogen was part of the nucleotides' nitrogenous bases.
- Bacteria of each culture were treated to release their DNA into a solution.
- The solution with DNA from both cultures was then centrifuged at high speed.
- The result was two bands of DNA, the band that was lower in solution contained the ^{15}N, the band that was higher contained the ^{14}N.

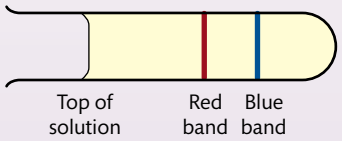


Figure 2.37 Meselson and Stahl's experiment.

- In Figure 2.37, you can see the two bands. The lower band had the heavier nitrogen, ^{15}N.
- This first tube represented a 'standard' to which future results could be compared.
- A new culture of *E. coli* was grown in the ^{15}N medium for many generations, to ensure all the DNA present was ^{15}N. A DNA sample was obtained and centrifuged. This became generation 0.
- At the same time as generation 0 was obtained, some of the bacteria were placed in a ^{14}N culture medium and allowed to grow for 20 minutes, which is the generation time for *E. coli* grown in optimal conditions.
- A sample was then taken, processed, and centrifuged to produce generation 1. This same process was continued so that four generations were obtained, each being processed and centrifuged.
- Figure 2.38 represents the results obtained.

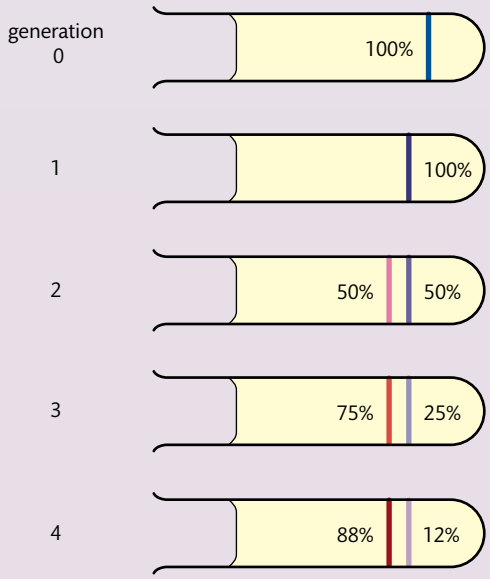


Figure 2.38 Meselson and Stahl's results.

Meselson and Stahl's experiment was performed half a century ago, but it employed techniques widely used in today's biological research. Meselson and Stahl made predictions based on a number of possible models. They then performed specially designed experiments to gather data to support one of these models.

Answer these questions from the results obtained.

(a) In semi-conservative replication, the new molecule of DNA has one strand from the original molecule and one new strand produced from nucleotides in the surrounding environment. How does generation 1 support this model?

(b) Why does generation 2 support the semi-conservative model?

The results of Meselson and Stahl's experiment are summarized in Figure 2.38. Notice the colours of the original strand of DNA and how one 'parent strand' becomes one of each of the new strands produced by replication.

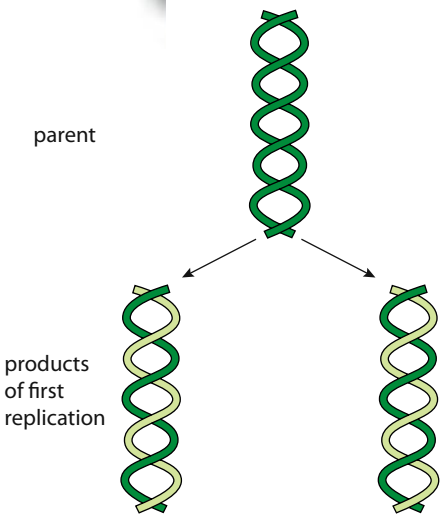
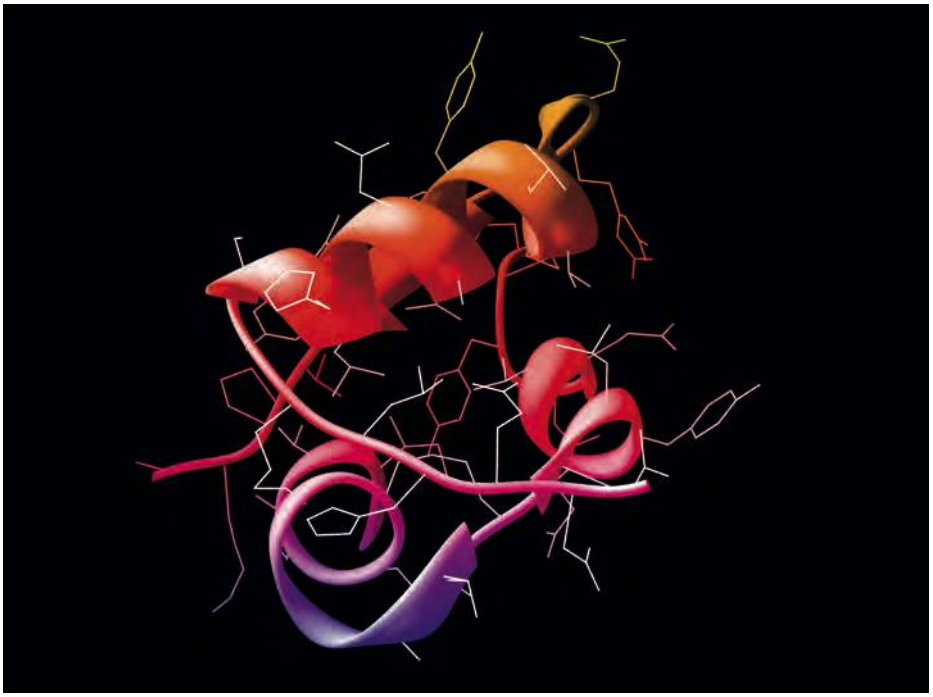


Figure 2.39 This figure demonstrates the general process of semi-conservative replication of DNA.

Protein synthesis

The control that DNA has over a cell is determined by a process called protein synthesis. In simple terms, DNA controls the proteins produced in a cell. Some of the proteins produced are enzymes. The production (or lack of production) of a particular enzyme can have a dramatic effect on the overall biochemistry of the cell. Thus DNA indirectly controls the biochemistry of carbohydrates, lipids, and nucleic acids with the production of enzymes.



This computer graphic shows an insulin molecule. Insulin is a protein hormone and is produced by protein synthesis.

Protein synthesis involves two major sets of reactions, transcription and translation. Both either produce or require a type of nucleic acid called RNA, which was discussed in Section 2.6.

Transcription produces RNA molecules

The sections of DNA that code for polypeptides are called genes. Any one gene is a specific sequence of nitrogenous bases found in a specific location in a DNA

molecule. Molecules of DNA are found within the confines of the nucleus, yet proteins are synthesized outside the nucleus in the cytoplasm. This means that there has to be an intermediary molecule that carries the message of the DNA (the code) to the cytoplasm where the enzymes, ribosome, and amino acids are found. This intermediary molecule is called messenger RNA (mRNA).

The nucleoplasm (fluid in the nucleus) contains free nucleotides, as mentioned earlier. In addition to the free nucleotides used for DNA replication, the nucleoplasm also contains free RNA nucleotides. Each of these is different from the DNA counterpart, because RNA nucleotides contain the sugar ribose not deoxyribose. Another major difference is that no RNA nucleotides contain thymine; instead there is a nitrogenous base unique to RNA, called uracil.

The transcription process

The process of transcription begins when an area of DNA of one gene becomes unzipped (see Figure 2.40). This is very similar to the unzipping process involved in DNA replication, but in this case only the area of the DNA where the particular gene is found is unzipped. The two complementary strands of DNA are now single-stranded in the area of the gene. Recall that RNA (which includes mRNA) is a single-stranded molecule. This means that only one of the two strands of DNA will be used as a template to create the mRNA molecule. An enzyme called RNA polymerase is used as the catalyst for this process.

As RNA polymerase moves along the strand of DNA acting as the template, RNA nucleotides float into place by complementary base pairing. The complementary base pairs are the same as in double-stranded DNA, with the exception that adenine on the DNA is now paired with uracil on the newly forming mRNA molecule. Consider the following facts concerning transcription:

- only one of the two strands of DNA is 'copied,' the other strand is not used
- mRNA is always single-stranded and shorter than the DNA that it is copied from, as it is a complementary copy of only one gene
- the presence of thymine in a molecule identifies it as DNA (the presence of deoxyribose is another clue)
- the presence of uracil in a molecule identifies it as RNA (the presence of ribose is another clue).

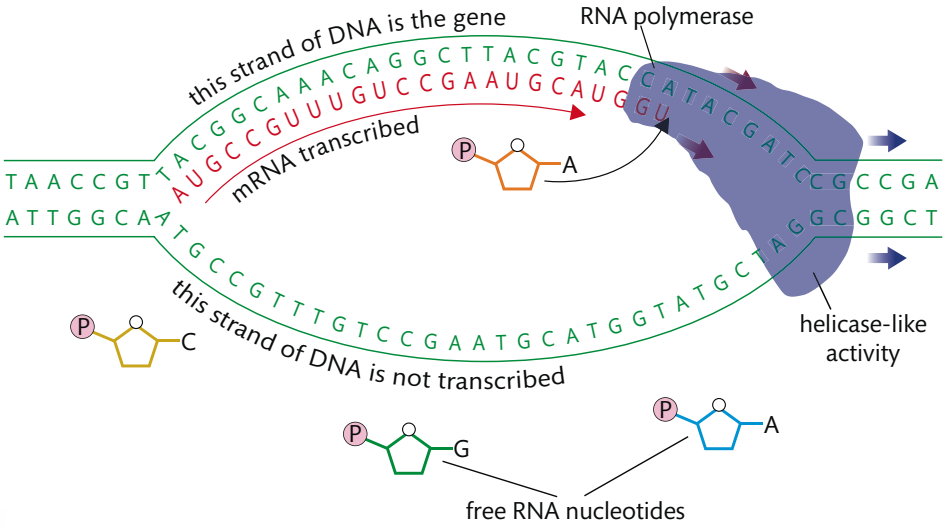


Figure 2.40 Transcription (synthesis of an RNA molecule). RNA polymerase has helicase-like activity as it plays a role in opening the DNA double helix. It also catalyses the addition of free RNA nucleotides to the growing mRNA strand.

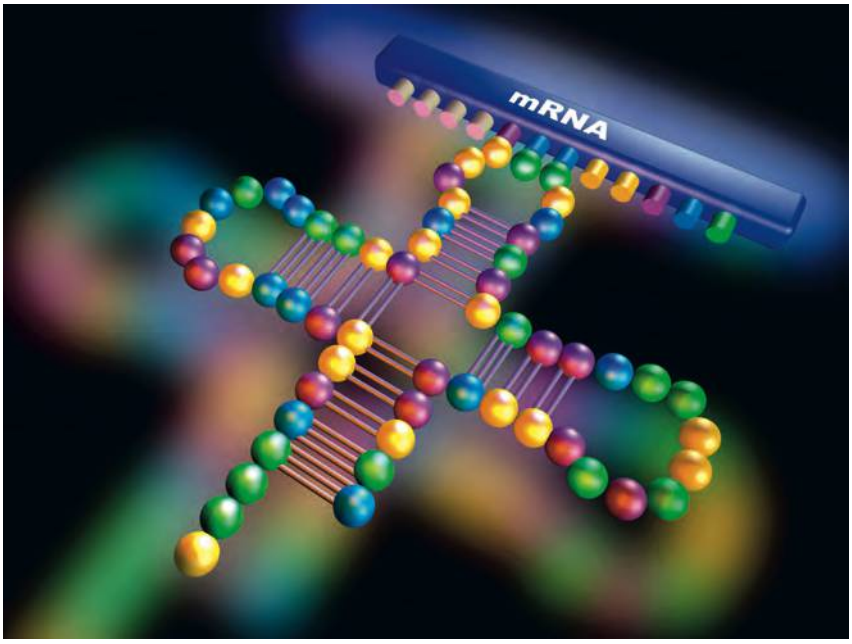
The genetic code is written in triplets

DNA triplet → (transcription) → mRNA (codon)

The mRNA molecule produced by transcription represents a complementary copy of one gene of DNA. The sequence of mRNA nucleotides is the transcribed version of the original DNA sequence. This sequence of nucleotides making up the length of the mRNA is typically enough information to make one polypeptide. As you will recall, polypeptides are composed of amino acids covalently bonded together in a specific sequence. The message written into the mRNA molecule is the message that determines the order of the amino acids. Researchers found experimentally that the genetic code is written in a language of three bases. In other words, a set of three bases contains enough information to code for one of the 20 amino acids. Any set of three bases that determines the identity of one amino acid is called a triplet. When a triplet is found in an mRNA molecule, it is called a codon or codon triplet. This is shown in the model below.

Translation results in the production of a polypeptide

There are three different kinds of RNA molecule. They are all single-stranded and each is transcribed from a gene (a section of DNA).



In this model, you can see mRNA (upper right) and tRNA (the clover shape). The amino acid that would be bonded to the tRNA is not shown.

Here is a quick summary of each RNA type:

- mRNA, messenger RNA, as described above, each mRNA is a complementary copy of a DNA gene and has enough genetic information to code for a single polypeptide
- rRNA, ribosomal RNA, each ribosome is composed of rRNA and ribosomal protein
- tRNA, transfer RNA, each type of tRNA transfers one of the 20 amino acids to the ribosome for polypeptide formation.

Figure 2.41 shows a typical tRNA molecule. Notice that the three bases in the middle loop are called the anticodon bases, and they determine which of the 20 amino acids is attached to the tRNA.

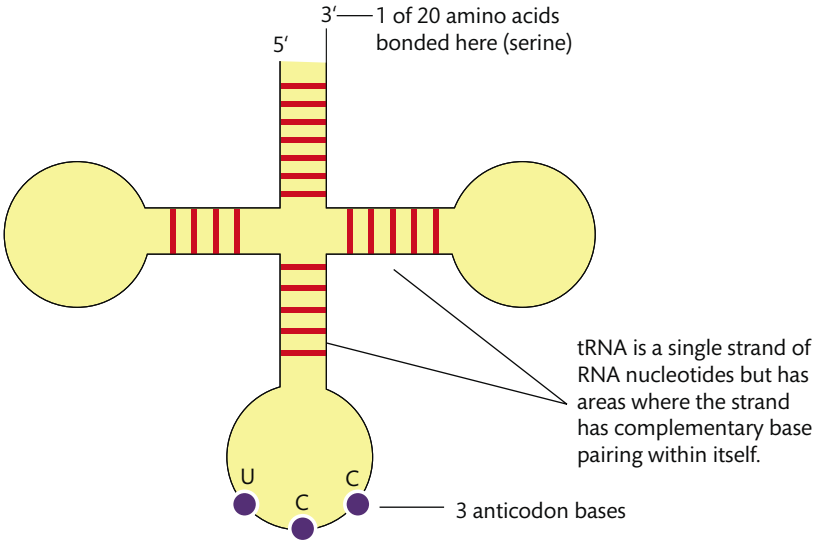


Figure 2.41 Structure of a tRNA 3′ molecule.

Once an mRNA molecule has been transcribed, the mRNA detaches from the single-strand DNA template and floats free in the nucleoplasm. At some point, the mRNA will float through one of the many holes in the nuclear membrane (nuclear pores) and will then be in the cytoplasm.

The translation process

The mRNA will locate a ribosome and align with it, so that the first two codon triplets are within the boundaries of the ribosome.

A specific tRNA molecule now floats in: its tRNA anticodon must be complementary to the first codon triplet of the mRNA molecule. Thus the first amino acid is brought into the translation process. It is not just any amino acid: its identity was originally determined by the strand of DNA that transcribed the mRNA being translated. While the first tRNA 'sits' in the ribosome holding the first amino acid, a second tRNA floats in and brings a second (again specific) amino acid. The second tRNA matches its three anticodon bases with the second codon triplet of the mRNA. As you can see in Figure 2.42, two specific amino acids are now being held side by side. An enzyme then catalyses a condensation reaction between the two amino acids, and the resulting covalent bond between them is called a peptide bond.

The next step in the translation process involves breaking the bond between the first tRNA molecule and the amino acid that it transferred in. This bond is no longer needed, as the second tRNA is currently bonded to its own amino acid, and that amino acid is covalently bonded to the first amino acid. The first tRNA floats away into the cytoplasm and invariably reloads with another amino acid of the same type. The ribosome that has only one tRNA in it now moves one codon triplet down the mRNA molecule. This, in effect, puts the second tRNA in the ribosome position that the first originally occupied, and creates room for a third tRNA to float in, bringing with it a third specific amino acid. The process now becomes repetitive: as another

The process of producing proteins utilizes a DNA code that is universal in all organisms. Because of this, researchers have successfully inserted the human gene that codes for the production of human insulin into bacteria. The result of this is bacteria that produce human insulin that can be used to treat humans with diabetes.

peptide bond forms, the ribosome moves on by another triplet, and so on. The process continues until the ribosome gets to the last codon triplet. The final codon triplet will be a triplet that does not act as a code for an amino acid, instead it signals 'stop' to the process of translation. The entire polypeptide breaks away from the final tRNA molecule, and becomes a free-floating polypeptide in the cytoplasm of the cell.

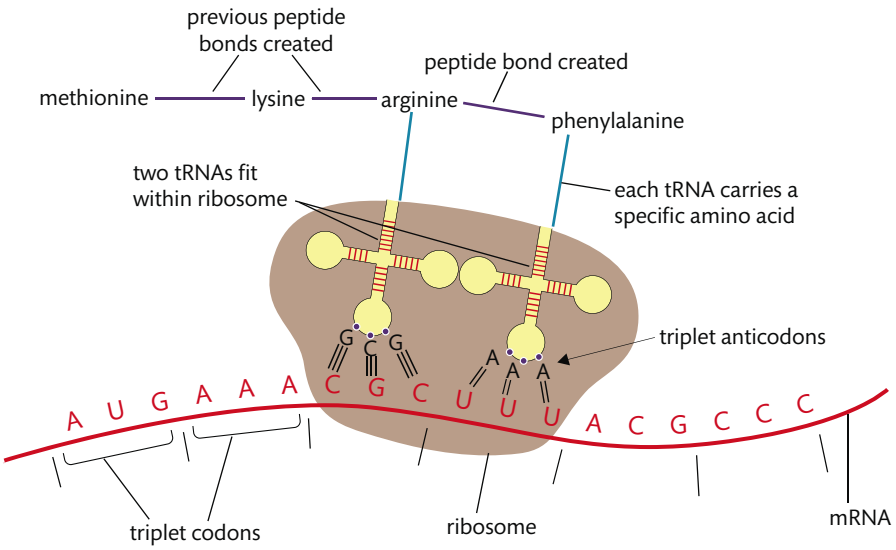


Figure 2.42 Events of translation (synthesis of a polypeptide).

Polymerase chain reaction and *Taq* DNA polymerase

Polymerase chain reaction, also known as PCR, was developed in the 1970s. It is a means by which DNA replication can be carried out artificially in a laboratory setting. However, it can only replicate rather short segments of DNA. By replicating DNA segments, scientists can produce huge numbers of these segments to study and analyse. It is often used in forensic situations when only a limited amount of the original DNA has been recovered at a crime scene.

An enzyme is used in PCR that is stable at relatively high temperatures. This enzyme was discovered in 1985 from a bacterium called *Thermus aquaticus* (*Taq*). This bacterium occurs naturally in hot springs, and its enzymes are not denatured at high temperatures, including the specific DNA polymerase that it possesses. This DNA polymerase has been named *Taq* polymerase and its use has greatly increased the number of discoveries in the field of gene technology.

Exercises

17 What type of bonds does helicase act upon?

18 What is the difference between a codon and a triplet?

19 What are the two major sets of reactions in protein synthesis?

20 What are the three major parts of all nucleotides?

TOK Who should decide how fast and how far humans should go with our study of DNA and the technology that is rapidly emerging?

To learn more about DNA replication and transcription and to find a codon chart, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.7.

CHALLENGE YOURSELF

12 Imagine that an mRNA leaves the nucleus of a eukaryotic cell with the following base sequence: AUGCCCCGCACGUUUCC AAGCCCCGGG.

Find an mRNA codon chart and answer the following.

(a) Determine in sequence the amino acids that are coded for by the above mRNA molecule.

(b) Determine the DNA code sequence that gave rise to the above mRNA codons.

(c) What would the amino acid sequence be if the first cytosine of the mRNA molecule was replaced with a uracil? (This would be the result of a change occurring in the DNA molecule that transcribed this mRNA.)

NATURE OF SCIENCE

Assessing the ethics of scientific research: the use of invertebrates in respirometer experiments has ethical implications.

2.8 Cell respiration

Understandings:

- Cell respiration is the controlled release of energy from organic compounds to produce ATP.
- ATP from cell respiration is immediately available as a source of energy in the cell.
- Anaerobic cell respiration gives a small yield of ATP from glucose.
- Aerobic cell respiration requires oxygen and gives a large yield of ATP from glucose.

Applications and skills:

- Application: Use of anaerobic cell respiration in yeasts to produce ethanol and carbon dioxide in baking.
- Application: Lactate production in humans when anaerobic respiration is used to maximize the power of muscle contractions.
- Skill: Analysis of results from experiments involving measurement of respiration rates in germinating seeds or invertebrates using a respirometer.

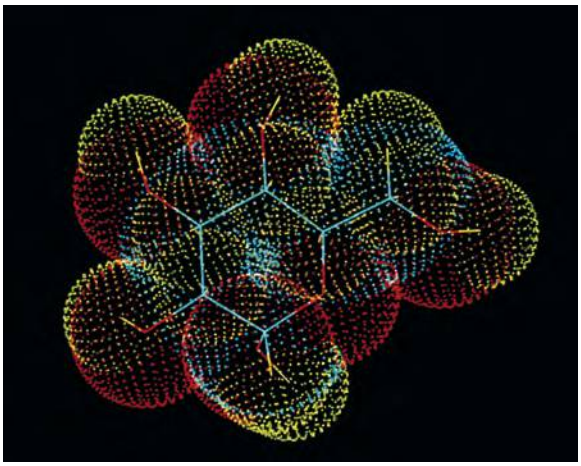
Guidance

- *Details of the metabolic pathways of cell respiration are not needed but the substrates and final waste products should be known.*
- *There are many simple respirometers that could be used. Students are expected to know that an alkali is used to absorb carbon dioxide, so reductions in volume are due to oxygen use. Temperature should be kept constant to avoid volume changes due to temperature fluctuations.*

Cell respiration is used by all cells to produce ATP

Organic molecules contain energy in their molecular structures. Each covalent bond in a glucose, amino acid, or fatty acid represents stored chemical energy. When we burn wood in a fire, we are releasing the stored chemical energy in the form of heat and light. Burning is the release of chemical energy called rapid oxidation.

Cells break down (or metabolize) their organic nutrients by slow oxidation. A molecule, such as glucose, is acted on by a series of enzymes. The function of these enzymes is to catalyse a sequential series of reactions in which the covalent bonds are broken (oxidized) one at a time. Each time a covalent bond is broken, a small amount of energy is released. The ultimate goal of releasing energy in a controlled way is to trap the released energy in the form of ATP molecules. If a cell does not have glucose available, other organic molecules may be substituted, such as fatty acids or amino acids.



This is a computer graphic of glucose. The backbone of the molecule is shown in stick form. The spheres represent the relative sizes of the individual atoms ($C_6H_{12}O_6$).

Glycolysis is the first step in the cell respiration process

Assuming that glucose is the organic nutrient being metabolized, all cells begin the process of cell respiration in the same way. Glucose enters a cell through the plasma membrane and floats in the cytoplasm. An enzyme modifies the glucose slightly, then a second enzyme modifies this molecule every more. This is followed by an entire series of reactions that ultimately cleaves the 6-carbon glucose into two 3-carbon molecules. Each of these 3-carbon molecules is called pyruvate. Some, but certainly not all, of the covalent bonds in the glucose are broken during this series of reactions. Some of the energy that is released from the breaking of these bonds is used to form a small number of ATP molecules. Notice in Figure 2.43 that two ATP molecules are needed to begin the process of glycolysis and a total of four ATP molecules are formed. This is referred to as a net gain of two ATP (a gain of four ATP minus the two ATP needed at the start).

Some cells use anaerobic respiration for ATP production

The term 'cell respiration' refers to a variety of biochemical pathways that can be used to metabolize glucose. All of the pathways start with glycolysis. In other words, glycolysis is the metabolic pathway that is common to all organisms on Earth. Some organisms derive their ATP completely without the use of oxygen and are referred to as anaerobic. The breakdown of organic molecules for ATP production in an anaerobic way is also called fermentation. There are two main anaerobic pathways, which will be discussed here separately: alcoholic fermentation and lactic acid fermentation.

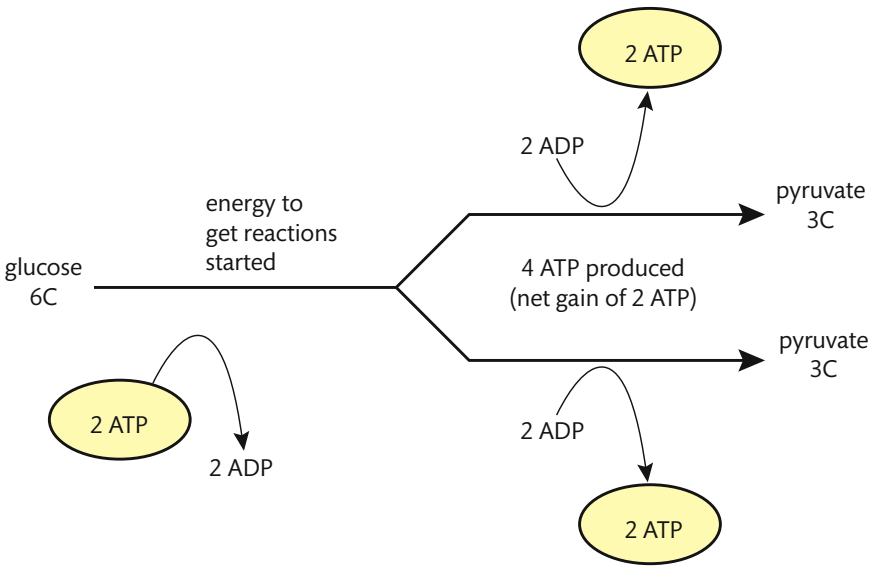


Figure 2.43 A simplified version of the events of glycolysis.

Most types of yeast are facultatively anaerobic, which means they only carry out alcoholic fermentation when oxygen is not available. If oxygen is present they actually carry out a different type of respiration, in which ethanol and carbon dioxide are not produced. Yeast cells are eukaryotic and do possess mitochondria.

Alcoholic fermentation

Yeast is a common single-cell fungus that uses alcoholic fermentation for ATP generation when oxygen is not present (see Figure 2.44). You will recall that all organisms use glycolysis to begin the cell respiration sequence. Thus yeast cells take in glucose from their environment and generate a net gain of two ATP by glycolysis. The organic products of glycolysis are always two pyruvate molecules. Yeast then converts both of the 3-carbon pyruvate molecules to molecules of ethanol. Ethanol is a 2-carbon molecule, so a carbon atom is 'lost' in this conversion. The 'lost' carbon atom is given off in a carbon dioxide molecule. Both the ethanol and carbon dioxide that are produced are waste products from the yeast and are simply released into the environment. Bakers' yeast is added to bread products for baking because the generation of carbon dioxide helps the dough to rise. It is also common to use yeast in the production of ethanol as alcohol to be drunk.

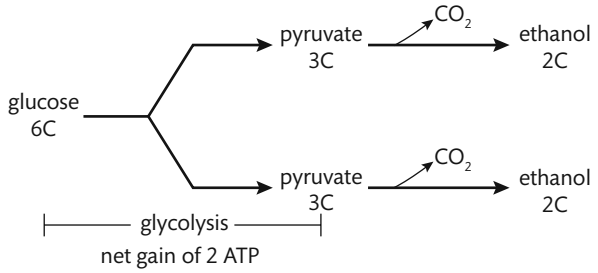


Figure 2.44 A simplified version of the events of alcoholic fermentation.

All alcohol that is sold to be drunk is ethanol. Beer, wine, and spirits contain different proportions of ethanol, plus other ingredients for flavouring.

Lactic acid fermentation

Organisms that normally use a cell respiration pathway that involves oxygen sometimes find themselves in a metabolic situation where they cannot supply enough oxygen to their cells. A good example of this is a person exercising beyond his or her normal pattern or routine. In this situation, the person's pulmonary and cardiovascular systems (lungs and heart) supply as much oxygen to the body's cells as is physically possible. If the person's exercise rate then exceeds his or her body's capacity to supply oxygen, at least some of the glucose entering into cell respiration will follow the anaerobic pathway called lactic acid fermentation. See Figure 2.45.

Once again, recall that glycolysis is used by all cells to begin the cell respiration sequence. Also remember that glycolysis:

- takes place in the cytoplasm
- results in the net gain of two ATP molecules per glucose molecule
- results in the production of two pyruvate molecules.

Cells that are aerobic normally take the two pyruvate molecules and metabolize them further in an aerobic series of reactions. But if cell is not getting a sufficient amount of oxygen for the aerobic pathway, i.e. is in a low-oxygen situation, excess pyruvate molecules are converted into lactic acid molecules. Like pyruvate, lactic acid molecules are 3-carbon molecules, so there is no production of carbon dioxide. What benefit does this serve? Lactic acid fermentation allows glycolysis to continue with a small gain of ATP in addition to the ATP that is generated through the aerobic pathway.

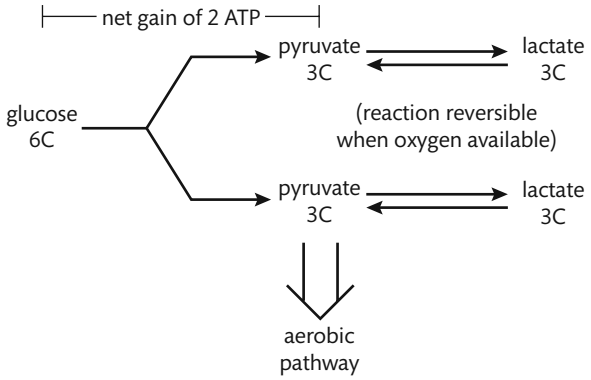
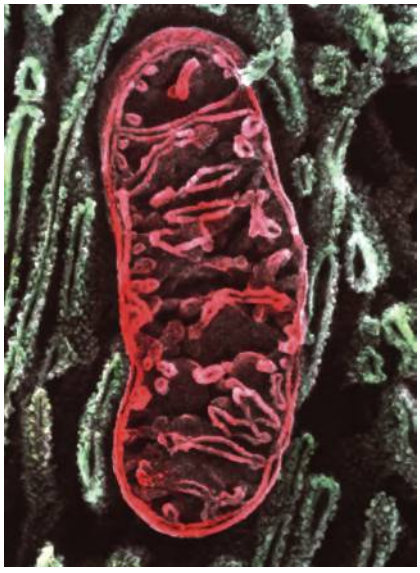


Figure 2.45 A simplified version of the events of lactic acid fermentation.

Aerobic cell respiration is the most efficient pathway

Cells that have mitochondria usually use an aerobic pathway for cell respiration. This pathway also begins with glycolysis, and thus has a net gain of two ATP as well as generating two pyruvate molecules. The two pyruvate molecules then enter a mitochondrion and are metabolized further.



This high-resolution, false-colour SEM shows a single mitochondrion. Any cell containing mitochondria uses aerobic cell respiration as its primary cell respiration pathway.

Each pyruvate first loses a carbon dioxide molecule and becomes a molecule known as acetyl-CoA. Each acetyl-CoA molecule enters into a series of reactions called the Krebs cycle. During this series of reactions, two more carbon dioxide molecules are produced from each original pyruvate molecule that entered it. The Krebs cycle is said to be a cycle because it is a series of chemical reactions that begin and end with the same molecule. This reacquisition of the beginning molecule allows this series of chemical reactions to be repeated over and over again (see Figure 2.46).

Some ATP is generated directly during the Krebs cycle and some is generated indirectly through a later series of reactions directly involving oxygen. Aerobic cell respiration breaks down (or completely oxidizes) a glucose molecule and the end-products are carbon dioxide and water plus a much higher number of ATP molecules than anaerobic respiration yields.

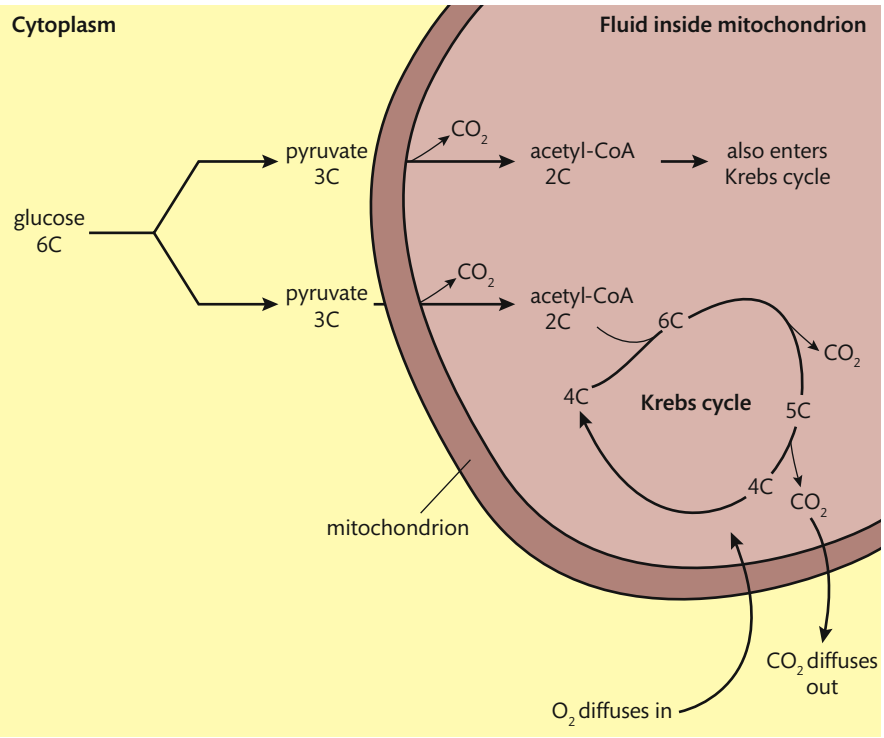


Figure 2.46 Aerobic cell respiration. Notice that the 4C molecule of the Krebs cycle combines with the 2C molecule called acetyl-CoA. The resulting 6C molecule then goes through a series of reactions in which two carbons are lost in the form of carbon dioxide. This restores the 4C molecule that can begin the cycle all over again.

Worked example

Respirometers are devices used to measure an organism's rate of respiration by measuring the oxygen rate of exchange. They are sealed units in which any carbon dioxide produced is absorbed by an alkali such as soda lime or potassium hydroxide. Absorbing the carbon dioxide allows an accurate measurement of oxygen exchange. These devices may work at a cellular level or at a whole-organism level. Look at the graph and answer the questions. The *y*-axis of the graph represents the relative amount of oxygen used.

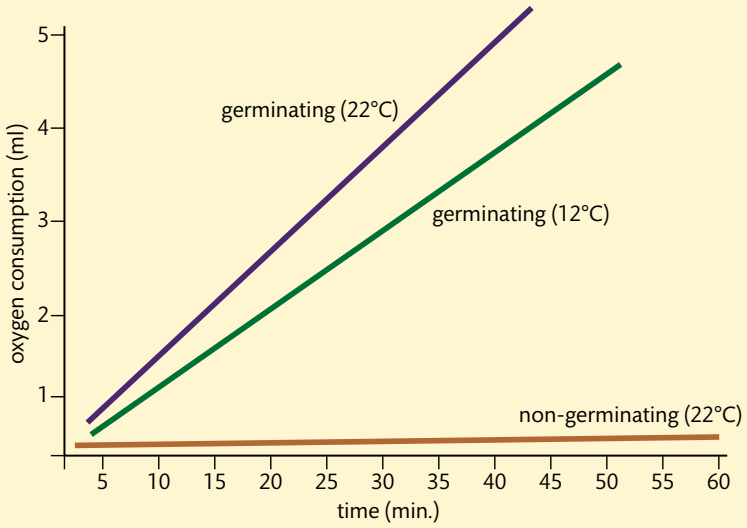


Figure 2.47 Oxygen consumption by germinating and non-germinating pea seeds at 12°C and 22°C.

1 In the germinating pea seeds, what type of respiration is occurring? What is the evidence for this answer?

2 Why is the oxygen consumption of non-germinating pea seeds very low?

3 Why would the germinating seeds show a greater oxygen consumption at 22°C than at 12°C?

4 Predict how the graph would look for non-germinating seeds at 12°C.

Solutions

1 Aerobic. There is a significant amount of oxygen consumption occurring.

2 They are not carrying out respiration and have a low metabolic rate.

3 At 22°C the rate of respiration is faster than at 12°C. Therefore, there is a greater oxygen consumption at the higher temperature.

4 The line of the graph would be almost right on the non-germinating (22°C) line that exists now. A prediction that it would be just slightly lower is best.

NATURE OF SCIENCE

It is tempting to place invertebrates in respirometers to determine oxygen consumption. However, the use of invertebrates in such experiments has ethical implications. It is essential to refer to the IB animal experimental policy before carrying out any procedures on animals. A discussion of the ethics of animal use in respirometer experiments would be wise at this point.

Exercises

21 Which stage of cell respiration is common to all types of cell respiration?

22 Where does this stage of cell respiration occur in a cell?

23 Why does that make sense?

24 Why do we inhale oxygen and exhale carbon dioxide?

To learn more about aerobic cell respiration, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.8.

2.9 Photosynthesis

NATURE OF SCIENCE

Experimental design: controlling relevant variables in photosynthesis experiments is essential.

Understandings:

- Photosynthesis is the production of carbon compounds in cells using light energy.
- Visible light has a range of wavelengths, with violet the shortest wavelength and red the longest.
- Chlorophyll absorbs red and blue light most effectively, and reflects green light more than other colours.
- Oxygen is produced in photosynthesis from the photolysis of water.
- Energy is needed to produce carbohydrates and other carbon compounds from carbon dioxide.
- Temperature, light intensity, and carbon dioxide concentration are possible limiting factors on the rate of photosynthesis.

Applications and skills:

- Application: Changes to the Earth's atmosphere, oceans, and rock deposition due to photosynthesis.
- Skill: Drawing an absorption spectrum for chlorophyll, and an action spectrum for photosynthesis.
- Skill: Design of experiments to investigate the effect of limiting factors on photosynthesis.
- Skill: Separation of photosynthetic pigments by chromatograph.

Guidance

- *Students should know that visible light has wavelengths between 400 and 700 nm, but they are not expected to recall the wavelengths of specific colours of light.*
- *Water free of dissolved carbon dioxide for photosynthesis experiments can be produced by boiling and cooling water.*
- *Paper chromatography can be used to separate photosynthetic pigments but thin layer chromatography gives better results.*

Photosynthesis converts light energy into chemical energy

Plants and other photosynthetic organisms produce foods that start food chains. We count on the Sun as a constant energy source for both warmth and food production for all of our planet. However, the sunlight that strikes Earth must be converted into a form of chemical energy in order to be useful to all non-photosynthetic organisms. The most common chemical energy produced from photosynthesis is the molecule glucose. If you recall, glucose is also the most common molecule that organisms use for fuel in the process of cell respiration.

Plants use the pigment chlorophyll to absorb light energy

The vast majority of plant leaves appear green to our eyes. If you were able to zoom into leaf cells and look around, you would see that the only structures in a leaf that are actually green are the chloroplasts. Plants contain a variety of pigments in chloroplasts. The photosynthetic pigment that dominates in most plant species is the molecule chlorophyll.

Separation of photosynthetic pigments by chromatograph

Safety alerts: Fumes from the chemicals used in this procedure are dangerous. Use the chemicals in a well-ventilated area or under an exhaust or fume hood. Wear goggles and a lab apron throughout the procedure. Follow all your teacher's specific instructions.

As stated above, many plants contain a variety of pigments. A procedure known as paper chromatography can separate the pigments present in most modern plants. The pigment called chlorophyll *a* is the principal pigment. Chlorophyll *b*, carotenes, and xanthophylls act as accessory pigments by absorbing light at different wavelengths, and passing this energy on to chlorophyll *a* to be used in photosynthesis.

- Spinach, *Spinacia oleracea*, or kale, *Brassica oleracea*, leaves are recommended for this procedure. A chromatography solvent that consists of an organic solvent, such as a type of alcohol, acetone, or petroleum ether, will be used. Be very careful with the solvent, it is highly flammable and should be worked with under some type of a fume or exhaust hood. A strip of chromatography paper must be used for this lab as well.
- Place a line of pigment from the leaf on a strip of chromatography paper using a 'ribbed' coin. This line should be dark in colour and as thin as possible. Several repeated applications with the coin at the same place on the paper should result in a dark-coloured line.
- The paper, with a pencil mark where the pigment was placed, is then positioned inside a closed chromatography chamber filled with a shallow layer of chromatography solvent. This solvent layer should reach between the tip of the paper and the pencil line for the pigment. The paper is then placed in the closed chromatography chamber until the solvent comes to within 1–2 cm of the top of the paper.
- When the solvent has reached this position on the paper, remove the paper from the chamber, keeping all parts under the exhaust or fume hood, and immediately mark the position of the solvent line. Mark the positions of the different coloured pigments on the paper.
- Now calculate the R_f value for each of the separated pigments.

R_f refers to retention factor or relative mobility factor. R_f = distance moved by pigment/distance moved by solvent. Record your results below.

Pigment colour	Distance solvent moved	Distance pigment moved	R_f value
Carotene (orange)			
Xanthophylls (yellow)			
Light green (chlorophyll *a*)			
Green (chlorophyll *b*)			

Table 2.11 Lab results

1. Explain why the four pigments moved at different rates through the chromatography paper.
2. Would any leaf from any plant have each of the pigments that are present in spinach or kale? How would this affect the chromatograph of these different leaves?
3. A procedure known as thin layer chromatography would give even better results than chromatography paper. Research and explain the difference between thin layer chromatography and paper chromatography.

Plants make use of the same part of the electromagnetic spectrum that our eyes are able to see. We call this the visible portion of the spectrum. Sunlight is actually a mixture of different colours of light. You can see these colours when you let sunlight pass through a prism.

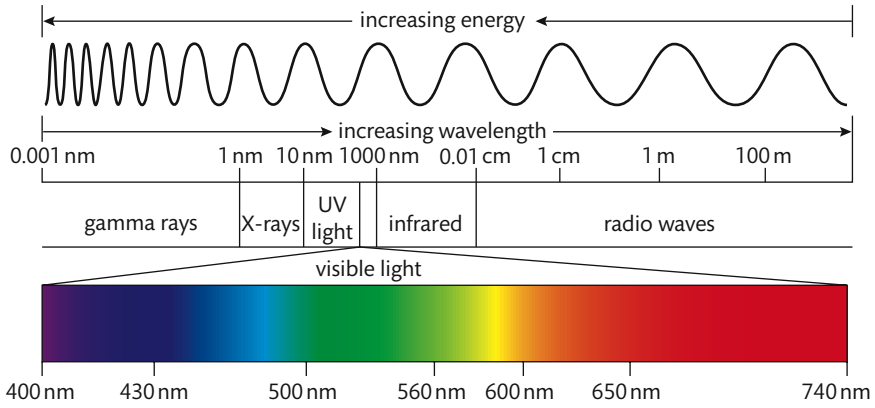
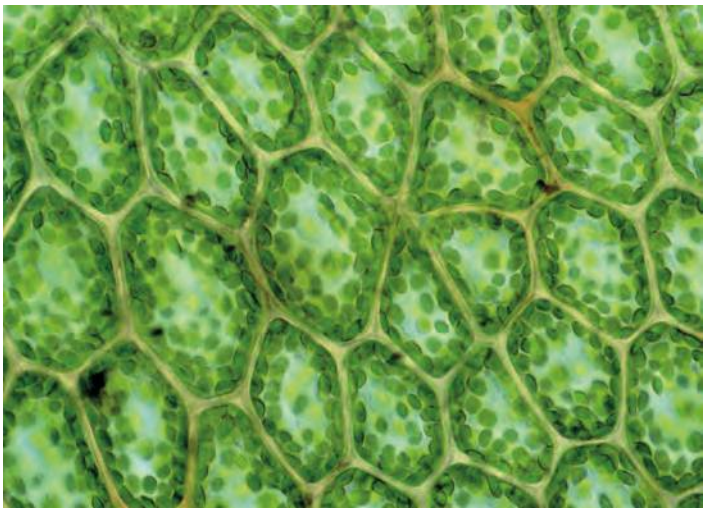


Figure 2.48 The electromagnetic spectrum. Notice that the visible light portion of this spectrum has colours with wavelengths of between 400 nm and 740 nm.



Inside each of these plant leaf cells are many green chloroplasts. Each chloroplast is loaded with chlorophyll.

The visible light spectrum includes many colours, but, for the purpose of considering how chlorophyll absorbs light energy, we are going to consider three regions of the spectrum:

- the red end of the spectrum
- the green middle of the spectrum
- the blue end of the spectrum.

Substances can do one of only two things when they are struck by a particular wavelength (colour) of light. They can:

- absorb that wavelength (if so, energy is being absorbed and may be used)
- reflect that wavelength (if so, the energy is not being absorbed and you will see that colour).

Worked example

You are walking outside with a friend who is wearing a red and white shirt. Explain why the shirt appears to be red and white.

Solution

Sunlight is a mixture of all of the wavelengths (colours) of visible light. When sunlight strikes the red pigments in the shirt, the blue and the green wavelengths of light are absorbed, but the red wavelengths are reflected. Thus, our eyes see red. When sunlight strikes the white areas of the shirt, all the wavelengths of light are reflected and our eyes and brain interpret the mixture as white.

Let's apply this information to how chlorophyll absorbs light for photosynthesis. Chlorophyll is a green pigment. This means that chlorophyll reflects green light and therefore must absorb the other wavelengths of the visible light spectrum. When a plant leaf is hit by sunlight, the red and blue wavelengths of light are absorbed by chlorophyll and used for photosynthesis. Almost all the energy of the green wavelengths is reflected, not absorbed.

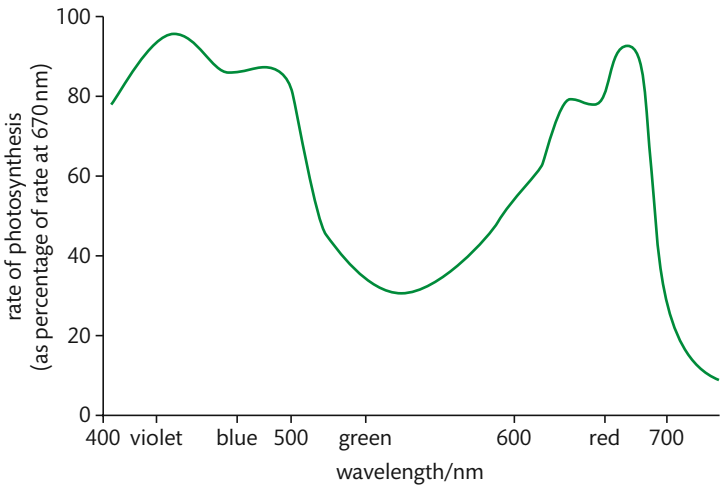


Figure 2.49 This action spectrum of photosynthesis indicates that most photosynthesis occurs in the blue and the red wavelength areas. Note the lower rate of photosynthesis with the green wavelength.

Photosynthesis occurs in two stages

Photosynthesis produces sugar molecules as a food source for the plant. Sugars, such as glucose, are held together by covalent bonds. It requires energy to create those covalent bonds, and the source of that energy can ultimately be traced back to the Sun.

The first stage of photosynthesis is a set of reactions that 'trap' light energy and convert it to the chemical energy of ATP. The second stage of photosynthesis is a set of reactions in which ATP is used to help bond carbon dioxide and water molecules together to create a sugar, such as glucose.

The first stage of photosynthesis

The first stage of photosynthesis is a set of reactions typically referred to as the light-dependent reactions (see Figure 2.50). In this set of reactions, chlorophyll (and other photosynthetic pigments) absorb light energy and convert that energy to a form of chemical energy, specifically ATP. In addition, light energy is used to accomplish a reaction that is called photolysis of water. In this reaction, a water molecule is split into its component elements: hydrogen and oxygen.

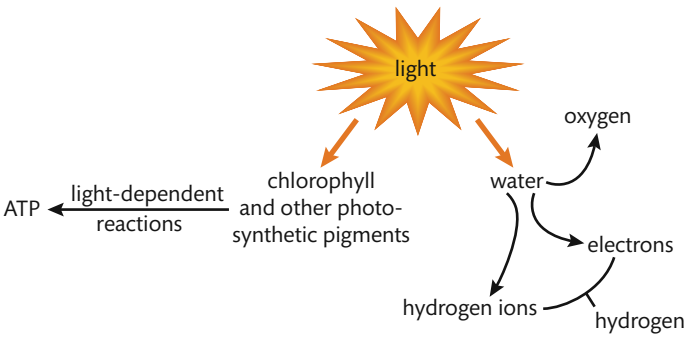


Figure 2.50 Functions of light during photosynthesis.

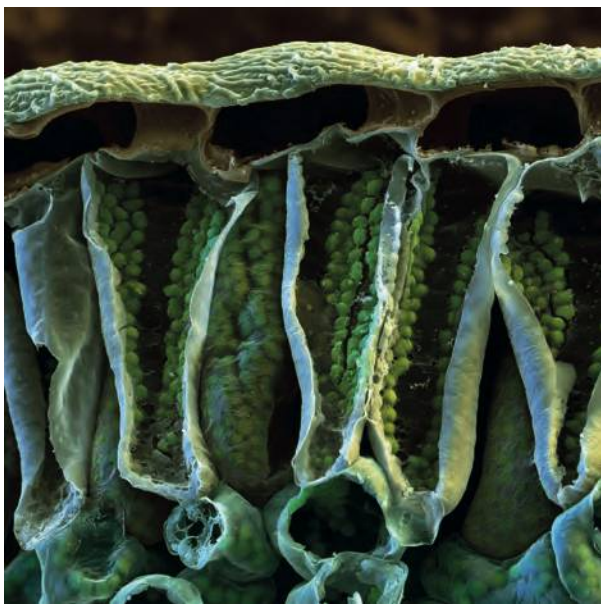
The oxygen that is split away due to the photolysis of water is typically released from the plant leaf as a waste product. From the plant's perspective, the useful products formed during this stage of photosynthesis are ATP and hydrogen.

The second stage of photosynthesis

The second stage of photosynthesis is a series of reactions collectively referred to as the light-independent reactions. ATP and hydrogen are used as forms of chemical energy to convert carbon dioxide and water into useful organic molecules for the plant. Glucose, a typical product of photosynthesis, is an organic molecule. It requires six inorganic carbon dioxide molecules to form one glucose molecule.

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

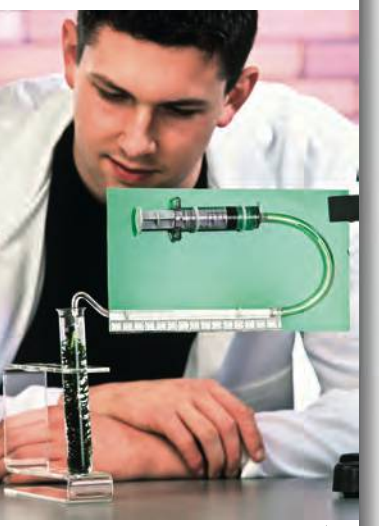
This conversion of an inorganic form of an element to an organic form is known as fixation. Therefore, photosynthesis can be described as a series of reactions in which carbon dioxide and water are fixed into glucose, and oxygen is produced as a by-product.



This is an SEM (with false colour added) of an upper leaf section. These cells are very active in photosynthesis, as is shown by the large number of chloroplasts.

The fixation reaction described above requires energy. The energy to create the glucose comes directly from the ATP and hydrogen created in the first stage of photosynthesis. Ultimately, this energy can be traced back to sunlight. It is also important to note that glucose is only one of the many possible organic molecules that can be formed from photosynthesis.

Measuring the rate of photosynthesis



This student is measuring oxygen produced by an aquatic plant. The rate of oxygen produced is a direct reflection of the rate of photosynthesis.

Look again at the summary reaction for photosynthesis:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$$

This balanced equation shows us that carbon dioxide molecules are reactants and oxygen molecules are products of photosynthesis. If you recall some of the information you learned earlier about cell respiration, you will see that the reverse is true for that process. In other words, for cell respiration oxygen is a reactant and carbon dioxide is a product.

At any given time of year, any one plant has a fairly consistent rate of cell respiration. Not only is this rate consistent throughout the day and night, it is also at a relatively low level. Plants need ATP for various biochemical processes, but the level is typically far lower than any animal needs.

The same consistency is not true regarding the rate of photosynthesis. The photosynthetic rate is highly dependent on many environmental factors, including the intensity of light and air temperature. During the daytime, especially on a warm sunny day, the rate of photosynthesis may be very high for a particular plant. If so, the rate of carbon dioxide taken in by the plant and the rate of oxygen released will also be very high. Because the plant is also carrying out cell respiration, a correction needs to be made for the carbon dioxide and oxygen levels. At night, the rate of photosynthesis may drop to zero. At that time, a particular plant may be giving off carbon dioxide and taking in oxygen to maintain its relatively low and consistent rate of cell respiration (see Figure 2.51).

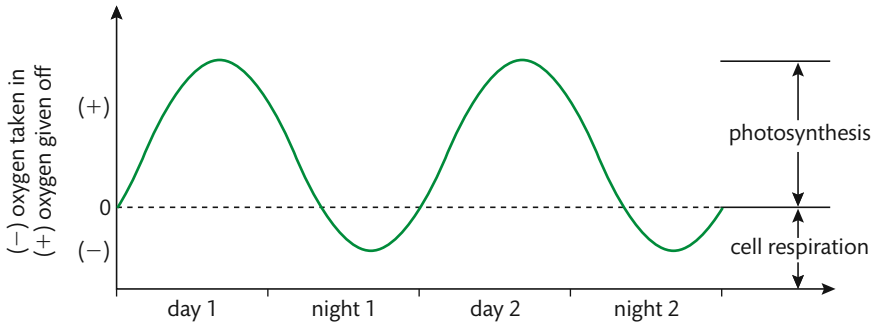


Figure 2.51 Graph showing the oxygen given off and taken in by a hypothetical plant over a 48-hour period. When the line intersect is at 0, the oxygen generated by photosynthesis is equal to the oxygen needed for cell respiration.

Measuring the rate of oxygen production or carbon dioxide intake is considered to be a direct measurement of photosynthetic rate as long as a correction is made for cell respiration. Another common method for measuring photosynthesis is to keep track of the change in biomass of experimental plants. However, the mass of plants is considered to be an indirect reflection of photosynthetic rate, as an increase or decrease in biomass may be caused by a whole variety of factors as well as the photosynthetic rate.

The effects of changing environmental factors on the rate of photosynthesis

Look now at the patterns that can be seen when three common environmental factors are varied, and how these factors are predicted to change the rate of photosynthesis in a generalized plant (Figures 2.52–2.54).

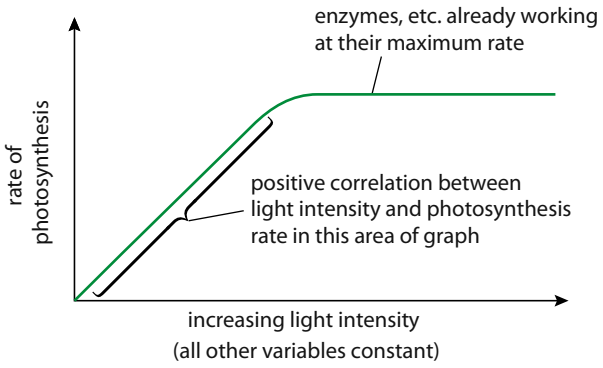


Figure 2.52 The effect of increasing light intensity on the rate of photosynthesis.

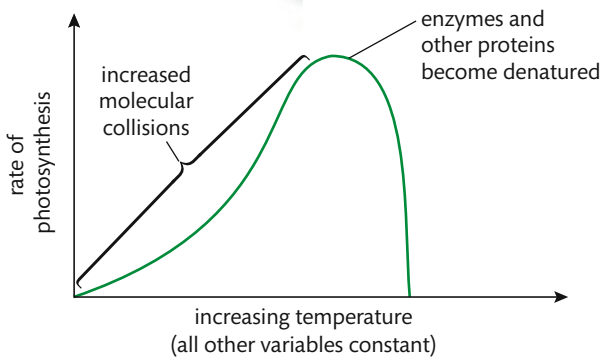


Figure 2.53 The effect of increasing temperature on the rate of photosynthesis.

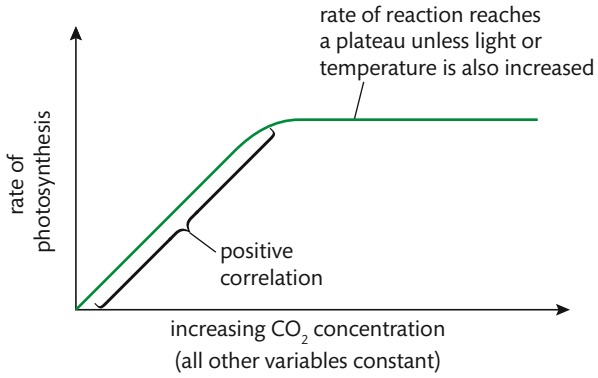


Figure 2.54 The effect of increasing carbon dioxide concentration on the rate of photosynthesis.

CHALLENGE YOURSELF

13 Many scientists have been involved in the development of the concept of limiting factors. They include Justus von Leibig, F. F. Blackmann, and Walter Taylor. A limiting factor is described as a factor that would most directly affect the rate of a physiological process. In photosynthesis, the limiting factor is the one that affects the rate of the photosynthetic process regardless of the effects of other factors. In many cases, it is the one factor that is in 'shortest' supply. Use Figures 2.52–2.54 to answer the following questions about photosynthesis and limiting factors.

(a) When examining the effect of light intensity on the rate of photosynthesis in Figure 2.52, why is the early part of the graph labelled as a positive correlation?

(b) In Figure 2.53, why does the denaturing of enzymes and other proteins at high temperatures dramatically lower the rate of photosynthesis?

(c) In Figure 2.54, what could possibly cause a change from the plateau shown to an increasing rate?

(d) Design a procedure to investigate the effect of one of the limiting factors mentioned above on the rate of photosynthesis. Some useful information to use in your planning is that water for photosynthesis experiments can be made to be free of dissolved carbon dioxide by boiling and then cooling it.

To learn more about photosynthesis, go to the hotlinks site, search for the title or ISBN, and click on Chapter 2: Section 2.9.

Exercises

25 Explain why a blue object appears to be blue to the human eye.

26 Explain why black surfaces (like tarmacadam and asphalt) get much hotter in sunlight than lighter surfaces (like stone and concrete).

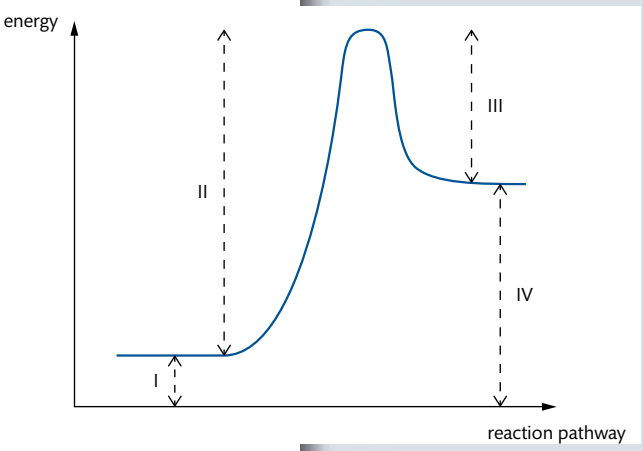
27 Plants produce sugars by photosynthesis. What do plants do with the sugars after that?

28 Why do most plants produce an excess of sugars in some months of the year?

Practice questions

1 Draw the basic structure of an amino acid, and label the groups that are used in peptide bond formation.

(*Total 4 marks*)



2 The graph to the left shows the energy changes in a chemical reaction.

What would happen to the changes in energy if this reaction was controlled by an enzyme?

A I would increase.

B II would decrease.

C I and IV would decrease.

D II and III would decrease.

(*Total 1 mark*)

3 What causes water to have a relatively high boiling point?

A Hydrogen bonds between water molecules.

B Hydrogen bonds between hydrogen and oxygen within water molecules.

C Cohesion between water molecules and the container in which the water is boiled.

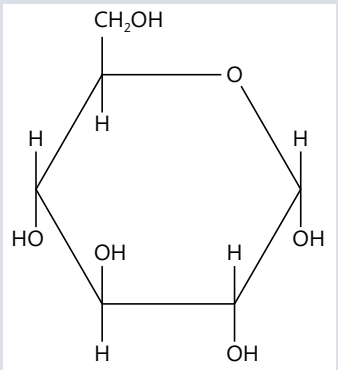
D Covalent bonds between hydrogen and oxygen within water molecules.

(*Total 1 mark*)

4 Outline the significance to organisms of the different properties of water.

(*Total 5 marks*)

5



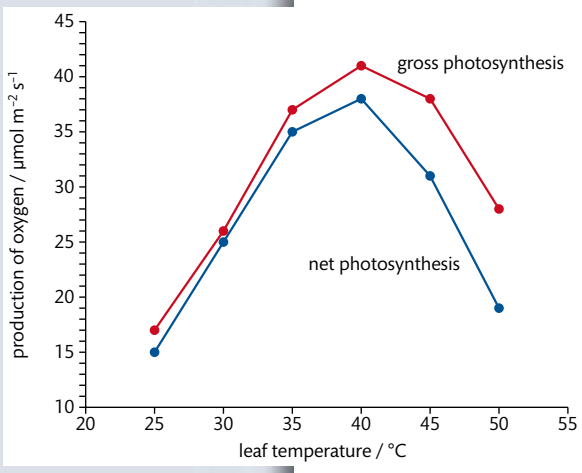
Which of the following terms correctly describe(s) the molecule above?

I Monosaccharide. II Glucose. III Component of triglyceride.

A I only. **B** I and II only. **C** II and III only. **D** I, II and III.

(*Total 1 mark*)

6 The effect of temperature on photosynthesis was studied in sweet orange, *Citrus sinensis*, using leaf discs. The production of oxygen was used to measure the rate of photosynthesis.
Gross photosynthesis refers to the sum of net photosynthesis and respiration. Net photosynthesis was calculated by subtracting the rate of respiration in the dark from gross photosynthesis.



Ribeiro et al. 2006

(a) Identify the optimum temperature for photosynthesis in this plant. (1)

(b) Determine the difference between gross photosynthesis and net photosynthesis at 40°C and 50°C. (2)

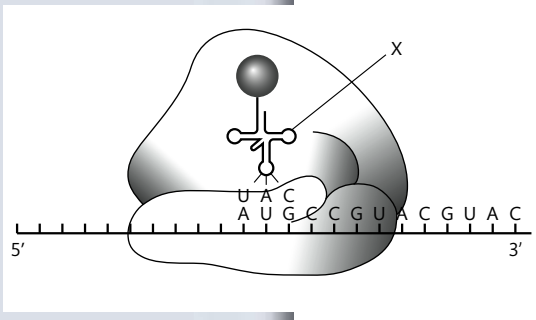
(c) Deduce what happens to the rate of respiration as the temperature increases between 40°C and 50°C. (1)

(d) (i) Describe the general pattern of change in photosynthesis in sweet orange as the temperature increases. (1)

(ii) Compare the effect of temperature on photosynthesis with the effect of temperature on respiration in sweet orange. (2)

(*Total 7 marks*)

7 What sequence of processes is carried out by the structure labelled X during translation?



A Combining with an amino acid and then binding to an anticodon.

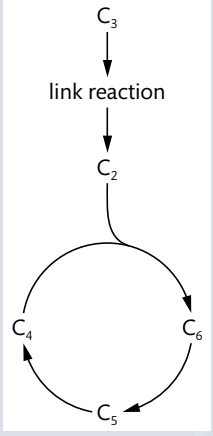
B Binding to an anticodon and then combining with an amino acid.

C Binding to a codon and then combining with an amino acid.

D Combining with an amino acid and then binding to a codon.

(Total 1 mark)

8 The diagram to the right shows part of the respiratory pathway. The number of carbon atoms in each molecule is indicated.



(a) (i) Label pyruvate and acetyl coenzyme A on the diagram. (1)

(ii) Indicate **two** places where decarboxylation occurs on the diagram. (1)

(iii) List **one** product other than carbon dioxide formed in this stage of respiration. (1)

(b) State precisely where in a cell this stage of respiration is occurring. (1)

(*Total 4 marks*)

03 Genetics

Essential ideas

3.1	Every living organism inherits a blueprint for life from its parents.
3.2	Chromosomes carry genes in a linear sequence that is shared by members of a species.
3.3	Alleles segregate during meiosis allowing new combinations to be formed by the fusion of gametes.
3.4	The inheritance of genes follows patterns.
3.5	Biologists have developed techniques for artificial manipulation of DNA, cells, and organisms.

One of the most famous experiments in biology was Gregor Mendel's pea-breeding investigation, which revealed important insights into the secrets of genetics.

- What will my first baby look like?
- Will my children be able to see the difference between red and green, even though I cannot?
- How can we find out who was at a crime scene by analysing their DNA?
- How can crops be genetically changed to improve their quality and quantity?
- Is it possible to clone humans?
- How many genes do I have?
- If I find a gene that has medical value, can I patent it and make money from my discovery?

In order to answer these questions, the mechanisms of genetics must be understood. Genetics is the science of how inherited information is passed on from one generation to the next using the genetic material of genes and deoxyribonucleic acid (DNA).

3.1 Genes

Understandings:

- A gene is a heritable factor that consists of a length of DNA and influences a specific characteristic.
- A gene occupies a specific position on a chromosome.
- The various specific forms of a gene are alleles.
- Alleles differ from each other by one or only a few bases.
- New alleles are formed by mutation.
- The genome is the whole of the genetic information of an organism.
- The entire base sequence of human genes was sequenced in the Human Genome Project.

Applications and skills:

- Application: The causes of sickle cell anaemia, including a base substitution mutation, a change to the base sequence of mRNA transcribed from it, and a change to the sequence of a polypeptide in haemoglobin.
- Application: Comparison of the number of genes in humans with other species.
- Skill: Use of a database to determine differences in the base sequence of a gene in two species.

NATURE OF SCIENCE

Developments in scientific research follow improvements in technology: gene sequencers are used for the sequencing of genes.

Guidance

- *Students should be able to recall one specific base substitution that causes glutamic acid to be substituted by valine as the sixth amino acid in the haemoglobin polypeptide.*
- *The number of genes in a species should not be referred to as genome size as this term is used for the total amount of DNA. At least one plant and one bacterium should be included in the comparison, and at least one species with more genes and one with fewer genes than a human.*
- *The GenBank® database can be used to search for DNA base sequences. The cytochrome c gene sequence is available for many different organisms and is of particular interest because of its use in reclassifying organisms into three domains.*
- *Deletions, insertions, and frame shift mutations do not need to be included.*

What is a gene?

Have you ever heard people say 'she looks just like her mum' or 'that kind of thing skips a generation'? Although those people might not have known it, they were talking about genetics.

CHALLENGE YOURSELF

1 Look at the list of characteristics below and think about which ones are determined by DNA and which are not. Are there some that can be influenced by both DNA and a person's environment? For example, most people who have inherited a light skin colour can darken their skin by tanning in the sun.

- Skin colour
- Freckles
- Number of fingers on each hand
- Blood type
- Colour blindness
- Sex (male/female)
- Ability to digest lactose
- Reflexes
- Type of ear wax (wet or dry)
- A scar from an accident
- Ability to speak
- Ability to speak Spanish
- Height
- Personality
- Intelligence

What about this: if a man had to have his left foot removed because of a war injury, would his future children be born with only one foot? Before scientists understood the mechanisms of genetics, it was believed that acquired characteristics could be passed on from one generation to the next. This idea has been refuted. The classic debate of nature versus nurture is a good topic for a Theory of knowledge discussion.

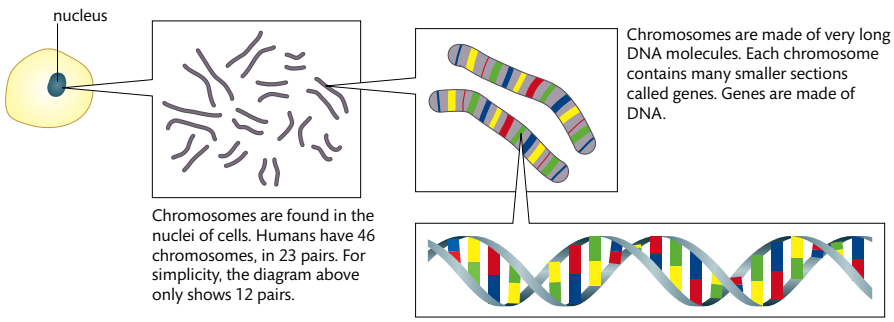


Figure 3.1 Zooming into a cell reveals where DNA is found.

Whenever a definition is given for a major concept in biology, in this instance the term 'gene', be sure to memorize its definition word for word. Such definitions have been phrased carefully so that all the important details are included.

Genes

A gene is a heritable factor that consists of a length of DNA and influences a specific characteristic. 'Heritable' means passed on from parent to offspring, and

'characteristic' refers to genetic traits such as your hair colour or your blood type. The estimated 21 000 genes that you possess are organized into chromosomes.

A gene is found at a particular locus on a chromosome

A gene for a specific trait occupies a corresponding place, called a locus (plural loci), on a chromosome (see Figure 3.2; there will be more about chromosomes in Section 3.2).

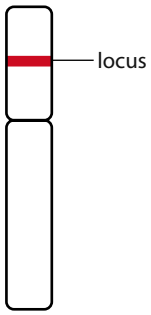


Figure 3.2 The locus is the specific position of a gene on a chromosome.

When geneticists map out the sequences of DNA, they carefully map the locus of each sequence. When further research reveals that a particular sequence controls a certain heritable factor, the locus of the gene is noted for further reference. For example, scientists now know that the locus of the gene controlling a protein called transducin that enables colour vision is found on chromosome 1. A mutation of this gene stops a person from being able to make the protein transducin properly, which is necessary to transmit information about colour from the eye to the brain; as a result, the person will not see in colour. This is an extremely rare genetic condition called complete achromatopsia. When we say 'the ability to see in colour is a genetic trait' we mean one of two things is happening with someone's DNA: either that person has the DNA code for making colour vision possible or the person does not have it. This is illustrated in Figure 3.3.

Where the gene is found:	Sequence of bases (starting at position 229):	Protein and trait:
Locus for transducin gene on chromosome 1	. . . CACCAG<u>C</u>AACCC . . .	Transducin protein synthesized, colour vision possible
	. . . CACCAG<u>T</u>AACCC . . .	Protein not formed correctly, colour vision not possible

Figure 3.3 The presence of a C or T makes a big difference in colour vision.

You will recall that you possess two copies of each gene in your body: one copy from your mother and one from your father. As a result, if you could look at the locus of the transducin gene on one of the two copies of your first chromosome, for example, you would find the same gene at the same locus on the other copy of chromosome 1. One copy would be the one your mother gave you and the other would be the copy your father gave you. Would those genes be identical? Not necessarily, because genes can come in different forms.

Alleles: versions of genes

Variations or versions of a gene are called alleles. An allele is one specific form of a gene, differing from other alleles by one or a few bases. In the example of transducin and colour vision above, a single base pair difference between the most common allele (with a C at position 235) and the rare mutated allele (with a T at position 235) is all that is takes to determine whether you can distinguish colours or not. These different forms allow for a single trait, such as the trait for the ability to see in colour, to have variants, in this example either colour or grey-scale vision. Another example of the difference between two alleles of the same trait is the difference that causes the genetic condition cystic fibrosis.

CHALLENGE YOURSELF

Table 3.1 Comparison of the number of genes in humans and other species

Organism	Scientific name	Number of bases	Number of genes
Virus (bacteriophage)	phiX174 *	5400	11
Bacterium	*Escherichia coli* (type K-12)	4639000	4377
Nematode (roundworm)	*Caenorhabditis elegans*	100292000	20000
Human	*Homo sapiens*	3000000000	21000
Asian rice	*Oryza sativa*	430000000	up to 56000
Baker's yeast	*Saccharomyces cerevisiae*	12495000	5770
Mouse-ear cress	*Arabidopsis thaliana*	135000000	25000
Fruit fly	*Drosophila melanogaster*	122654000	27407
Japanese canopy plant	*Paris japonica***	150000000000	Unknown

*First genome ever sequenced (in 1977).

**Largest plant genome sequenced so far.

2

(a) Which species has the largest number of genes?
(b) Which species has the smallest number of genes?
(c) Which species has the most similar number of genes to humans?
(d) Some people are tempted to say that the more genes an organism has, the more advanced it is. Discuss this idea: what kinds of arguments support it and what arguments refute it?

Cystic fibrosis

Maintaining a proper balance of fluids in the body is essential for good health. One such fluid is mucus, a thick, slippery, substance used in many parts of the body, including the lungs and intestines. A gene called *CFTR*, found on chromosome 7, plays a key role in the production of mucus. The standard version of this gene (the standard allele) allows a person's mucus-producing cells to function properly, whereas an allele generated by a mutation of the *CFTR* gene causes cystic fibrosis. People with this genetic condition produce abnormally excessive quantities of mucus in various organs and have difficulties with their respiratory and digestive systems, among other complications. In this example, the trait is for mucus production; one allele is for a balanced mucus production, the other for excessive mucus production that leads to cystic fibrosis. We will see later how to calculate the chances of a child inheriting this condition from his or her parents.

One base can make a big difference

From the sections on transcription and translation of DNA, you will remember how important it is for each letter in the genetic code to be in a specific place. If, for

whatever reason, one or more of the bases (A, C, G or T) is misplaced or substituted for a different base, the results can be dramatic. As we have seen with cystic fibrosis, the difference between one version of a gene and another (the mutated and non-mutated alleles of the *CFTR* gene) can mean the difference between healthy organs and organs hampered by an overproduction of mucus.

Another example of a change of bases can be seen in the gene *ABCC11*, which determines several things, one of them being whether or not the cerumen (ear wax) that you produce is wet or dry. Some people produce dry cerumen, which is flaky and crumbly with a grey colour, while others produce earwax that is more fluid and has an amber colour. The gene that determines this is on chromosome 16 and has two alleles: the G variant codes for dry cerumen, the A variant codes for wet cerumen. The allele containing G for wet earwax is much more common in European and African populations, while the allele containing A is much more common among Asians. Why is this of interest to geneticists? For one thing, it can reveal a lot about how populations have migrated and interbred in the past, but it can also reveal other things about our health. As curious as it may seem, the *ABCC11* gene is also partly responsible for the smell of underarm sweat, as well as the production of breast milk, and could potentially have a link to breast cancer. Most women probably would not care whether or not they have the gene for dry or fluid earwax, but if they could find out whether they had an allele that could reduce their chances of having breast cancer, they might be much more interested.

How are such differences in genes generated in populations? We will now look at how mutations work.

How new alleles are produced

Worked example

Look at the two sequences of DNA below, which are from the coding strand of a section of genetic information that helps in the formation of haemoglobin, found in red blood cells. Look carefully at the two sequences below. Identify the difference between the two and complete the phrase below.

DNA sequence 1: GTG CAC CTG ACT CCT GAG GAG

DNA sequence 2: GTG CAC CTG ACT CCT GTG GAG

'Codon number __ along the first sequence has the letter __ in position number __, whereas the codon in the same position in sequence 2 has the letter __ instead.'

Solution

Codon number 6 along the first sequence has the letter A in position number 2, whereas the codon in the same position in sequence 2 has the letter T instead.

Now look at the effect this has on the mRNA sequences produced from the template strand that is found opposite the coding strand when the DNA is unzipped for transcription:

mRNA sequence 1: GUG CAC CUG ACU CCU GAG GAG

mRNA sequence 2: GUG CAC CUG ACU CCU GUG GAG

Using Figure 3.4 and the mRNA sequences given on page 117, showing which codons are associated with which amino acids, fill in the names of the missing amino acids (a) to (h) in Figure 3.5.

Figure 3.4 Codons and their associated amino acids. For example, DNA coding for lysine is AAA.

Second base

First base	U	C	A	G	Third base
U	UUU, UUC – Phenylalanine UUA, UUG – Leucine	UCU, UCC, UCA, UCG – Serine	UAU, UAC – Tyrosine UAA, UAG – Stop codon Stop codon	UGU, UGC – Cysteine UGA Stop codon UGG Tryptophan	U C A G
C	CUU, CUC, CUA, CUG – Leucine	CCU, CCC, CCA, CCG – Proline	CAU, CAC – Histidine CAA, CAG – Glutamine	CGU, CGC, CGA, CGG – Arginine	U C A G
A	AUU, AUC, AUA – Isoleucine AUG Methionine start codon	ACU, ACC, ACA, ACG – Threonine	AAU, AAC – Asparagine AAA, AAG – Lysine	AGU, AGC – Serine AGA, AGG – Arginine	U C A G
G	GUU, GUC, GUA, GUG – Valine	GCU, GCC, GCA, GCG – Alanine	GAU, GAC – Aspartic acid GAA, GAG – Glutamic acid	GGU, GGC, GGA, GGG – Glycine	U C A G

Figure 3.5 Using Figure 3.4 and the mRNA sequences given on page 117, can you find the missing amino acids?

Sequence 1:	valine	–	histidine	–	(a)_____	–	(b)_____	–	(c)_____	–	(d)_____	–	glutamic acid
Sequence 2:	valine	–	histidine	–	(e)_____	–	(f)_____	–	(g)_____	–	(h)_____	–	glutamic acid

Solution

Sequence 1:	valine	–	histidine	–	leucine	–	threonine	–	proline	–	glutamic acid	–	glutamic acid
Sequence 2:	valine	–	histidine	–	leucine	–	threonine	–	proline	–	valine	–	glutamic acid

Figure 3.6 Is this what you found? We will need these sequences later when we explore sickle cell disease.

Notice how the error of only one letter in the original DNA code changed the composition of amino acids in sequence 2. This would change the composition and the structure of the resulting protein, in the same way that changing the shapes and compositions of some of the bricks used to build a house would change the shape (and therefore the structural integrity) of the house. This kind of change in the DNA code is produced by a mutation.

Mutations

A mutation is a random, rare change in genetic material. One type involves a change of the sequence of bases in DNA. If DNA replication works correctly, this should not happen (see Section 2.7). But nature sometimes makes mistakes. For example, the base thymine (T) might be put in the place of adenine (A) along the DNA sequence. When this happens, the corresponding bases along the messenger RNA (mRNA) are altered during transcription.

As we have seen with the example of cystic fibrosis, mutated genes can have a negative effect on a person's health. Sometimes, however, mutations can have a positive effect that is beneficial to an organism's survival.

On the left, a white-eyed mutant fruit fly, and on the right the kind of fruit fly typically found in nature, called the wild-type.

Are mutations good or bad for us?

LRP5 is a gene that helps immune system cells make a certain type of protein that acts as a receptor on their surfaces. Research indicates that this receptor is used by the human immunodeficiency virus (HIV) to infect the cells (see Section 6.3 for a description of HIV). People with a mutation of *LRP5* cannot make this receptor protein on their immune system's cells and, as a result, HIV cannot infect them. This means that people with a mutated allele of *LRP5* are naturally immune to HIV. Such a mutation is very rare in the human population.

A mutation that provides an individual or a species with a better chance for survival is considered to be a beneficial mutation, and there is a good chance that it will be passed on to the next generation. In contrast, mutations that cause disease or death are detrimental mutations, and they are less likely to be passed on to future generations, because they decrease the chances of an individual's survival. In addition to beneficial and harmful mutations, there are neutral mutations that do not have an effect on a species' survival.

When a mutation is successfully passed on from one generation to the next, it becomes a new allele: it is a new version of the original gene. This is how new alleles are produced. You and everyone you know possess many mutations. Whether they are harmful, beneficial or neutral depends on what they are and what kind of environment you need to survive in.

A gene to help digestion

For most of our existence, humans have been hunter-gatherers and our genes are generally well adapted for this lifestyle. Originally, as for all mammals, the only age at which we drank milk was when we were infants. By the time our ancestors reached adulthood, their bodies had stopped being able to digest milk; more precisely, humans could not break down the disaccharide in milk called lactose. This continues to be the case for most of the human population today: more than half of the human population has lactose intolerance and those people can only digest lactose in their infancy. In the past 10 000 years, however, many human populations have adopted an agricultural-based lifestyle, raising animals for milk and consuming dairy products on a daily

basis. In their genetic makeup, many agricultural societies show a higher frequency of the genetic code that allows humans to digest lactose throughout adulthood. From an evolutionary point of view, this advantage has increased humans' ability to survive harsh climatic conditions. As European human populations spread out and established populations outside Europe, notably in North America, they brought their lactose tolerance (and their livestock) with them.

Gene therapy is the process of taking a beneficial gene from a person who possesses it and putting it into a person who does not have it, but who needs it to stay healthy. The challenge is that it is very difficult to get the DNA into the sick person's cells. One way is to force the gene into the patient's cells using a virus to deliver it. Partly because of a lack of understanding of how to use viruses safely to deliver genes, the decision was made to stop all testing of gene therapy on human patients in the USA in 1999, when an 18-year-old patient died after a virus had been injected into his body. However, gene therapy trials are coming back, little by little, notably in helping blind children to regain their eyesight.

Who decides whether an experiment is safe? Is the loss of life for some patients participating in trials necessary in order to find a cure? If years of research had not been delayed because human trials had been stopped, wouldn't we have made much more progress by now in curing genetic diseases?

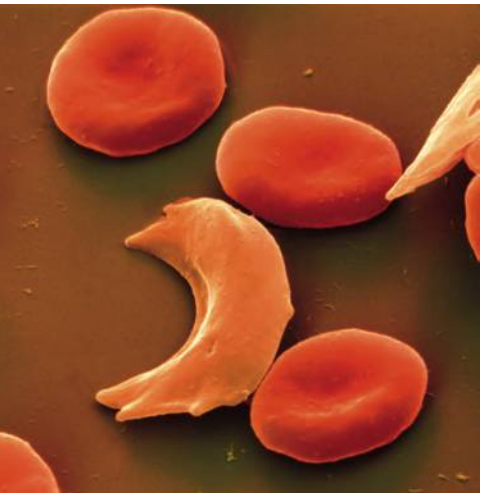
Base substitution mutation

The type of mutation that results in a single letter being changed is called a base substitution mutation. The consequence of changing one base could mean that a different amino acid is placed in the growing polypeptide chain. This may have little or no effect on the organism, or it may have a major influence on the organism's physical characteristics.

Sickle cell disease

In humans, a mutation is sometimes found in the gene that codes for haemoglobin in red blood cells. This mutation gives a different shape to the haemoglobin molecule. The difference leads to red blood cells that look very different from the usual flattened disc with a hollow in the middle.

The mutated red blood cell, with a characteristic curved shape, made its discoverers think of a sickle (a curved knife used to cut tall plants). The condition that results from this mutation is therefore called sickle cell disease, also known as sickle cell anaemia.



Three standard, disc-shaped red blood cells, and one sickle-shaped cell.

The kind of mutation that causes sickle cells is a base substitution mutation. If you look back at the two sequences given previously in the worked example on page 117, the first is for the section of the haemoglobin gene's DNA that codes for standard-shaped red blood cells, whereas the second sequence shows the mutation that leads to the sickle shape. In this case, one base is substituted for another so that the sixth codon in this sequence of haemoglobin, GAG, becomes GTG. As a result, during translation, instead of adding glutamic acid, which is the intended amino acid in the sixth position of the sequence, valine is added there instead. Again, refer back to the worked example to see this mutation.

Because valine has a different shape and different properties compared with glutamic acid, the shape of the resulting polypeptide chain is modified. As a result of this, the haemoglobin molecule has different properties that cause the complications associated with sickle cell disease.

The symptoms of sickle cell disease are weakness, fatigue, and shortness of breath. Oxygen cannot be carried as efficiently by the irregularly shaped red blood cells. In addition, the haemoglobin tends to crystallize within the red blood cells, causing them to be less flexible. The affected red blood cells can get stuck in capillaries, so blood flow can be slowed or blocked, a condition that is painful for the sufferer.

People affected by sickle cell anaemia are at risk of passing the mutated gene on to their offspring. From a demographics point of view, the mutated gene is mostly found in populations originating from West Africa or from the Mediterranean.

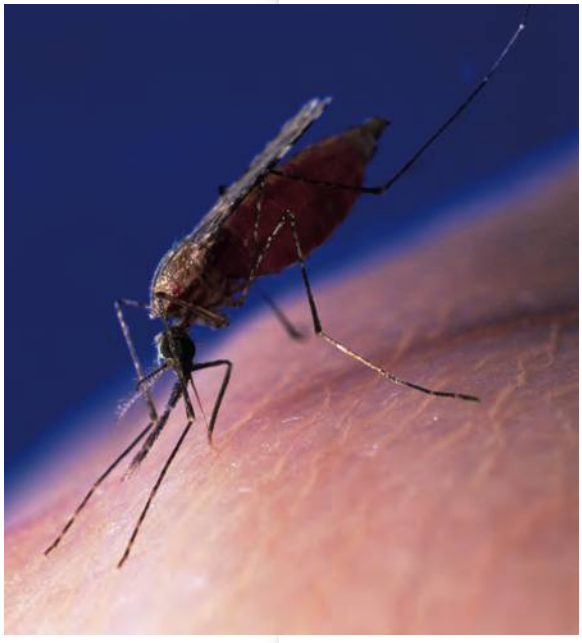
The advantages of sickle cell disease

Although sickle cell disease is a debilitating condition, those who have it are very resistant to malaria infection. Malaria is an infectious disease that occurs in tropical regions. A parasite called *Plasmodium* is transmitted to human blood by an infected female *Anopheles* mosquito feeding on the blood. The parasite attacks the person's red blood cells and produces symptoms of high fever and chills, and can result in death.

In terms of the shapes of human red blood cells, we all carry two copies of the gene for the shape of our red blood cells, one copy that we inherited from our mother and the other that we inherited from our father. People born with two copies for standard disc-shaped cell have only disc-shaped cells and are highly susceptible to malaria infection. People who have one gene that is for disc-shaped cells and one for sickle-shaped cells have what is called sickle cell trait. They have some sickle-shaped cells and some disc-shaped cells in their bloodstream but in most cases they do not suffer from anaemia. Anaemia is the result of low red blood cell levels and is characterized by a paleness of skin and low energy levels. People with sickle cell trait have a better resistance to malaria because of chemical imbalances that make the survival of *Plasmodium* in their blood more difficult. The insufficient quantities of potassium in sickle-shaped cells cause *Plasmodium* to die. Lastly, people who inherit a sickle cell gene from both their mother and their father can produce only sickle-shaped cells and suffer from severe anaemia that can sometimes be fatal. On the other hand, they have the highest resistance to malaria.

TOK

When we look at where sickle cell disease is most common in the world, there appears to be a significant overlap with the places where malaria occurs. Is this just a coincidence? Or is there a reason for this? Scientists and statistics experts often say that 'correlation does not mean causality', meaning that just because two things occur in the same place at the same time does not necessarily mean that one causes the other. How can we tell the difference between causality and correlation? The answer is that there must be some kind of mechanism that could explain how one could cause the other. From what you have read about sickle cell disease and malaria in this chapter, what do you think? Are they merely correlated or is there also causality?



Malaria can be transmitted by the female *Anopheles* mosquito, which is therefore one of the deadliest animals on Earth.

A genome

How do we know all that we do about genes? How do we know where they are and what they do? Before answering these questions, it is important to appreciate the point that, although we have made considerable progress in the past few decades, our maps of human chromosomes are still far from complete, and there are many DNA sequences for which we do not know the function. As an analogy, think of the maps produced by cartographers and explorers in the Middle Ages; many parts of the globe remained uncharted and had the words *terra incognita* (Latin for 'unknown land') inscribed on them.

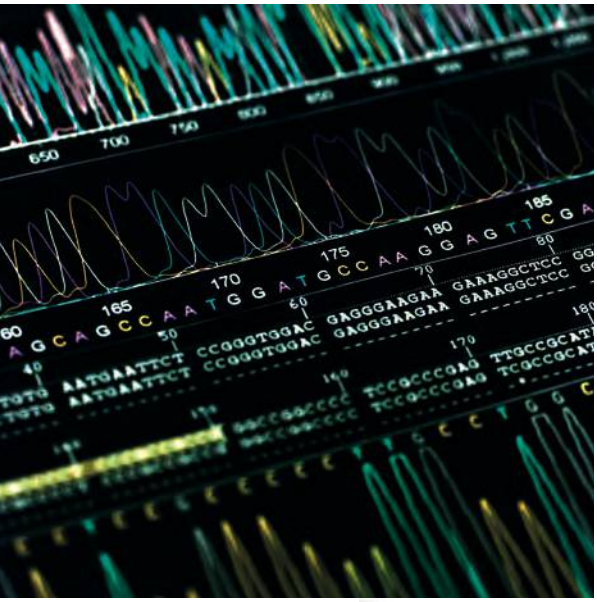
Sequencing DNA

In order to find out which gene does what, a list must be made showing the order of all the nucleotides in the DNA

code. Researchers use highly specialized laboratory equipment including sequencers to locate and identify sequences of bases. The complete set of an organism's base sequences is called its genome.

A short fragment of a sequence looks like this: GTGGACCTGACTCCTGAGGAG. Each letter represents one of the four bases in the DNA code. This short fragment contains seven codons with a total of 21 bases represented by letters. Now imagine 3 billion of those letters: what would that look like? If you printed out 3000 base letters per page, it would need 1 million pages, which would stack about 100 m high. That's an impressive quantity of information, especially considering that you can keep it all in the nucleus of a typical cell in your body.

The complete genomes of some organisms have been worked out. Among those organisms are the fruit fly, *Drosophila melanogaster*, and the bacterium, *Escherichia coli*, because these two organisms have been used extensively in genetics experiments for decades.



Computers are used to speed up the sequencing process.

How do geneticists work out the complete genome?

Many steps are necessary. Here is a summary of one way of doing it: the Sanger technique.

- Once a DNA sample has been taken, it is chopped up into fragments and copies are made of the fragments. A primer sequence is added to help start the process.
- To determine the sequence, a DNA polymerase enzyme attaches to one copy of the first fragment (let's call it fragment 1). Then it will start to add free nucleotides following the principle of complementary base pairing. Two kinds of nucleotides will be added.
- Some free nucleotides are standard ones and others are special dideoxynucleotide triphosphates (ddNTPs labelled ddA, ddT, ddC, and ddG in Figure 3.7) added as DNA chain terminators, meaning that when one is reached, the elongation of the strand is stopped. These have been previously marked with fluorescent markers to identify them. Sometimes the chain termination happens all the way at the end of fragment 1, but most of the time the process stops before it reaches the end. This process happens on each of the many copies of fragment 1.
- The result is a series of new strands, some dozens of bases long, others only a handful of bases long, and some that have all the bases of fragment 1.
- Now everything is ready for the sequencing: the multiple chains of varying lengths (each with a fluorescently marked end) are placed in order from longest to shortest. This is done using a technique called gel electrophoresis, which will be explained in Section 3.5.
- To recognize each letter, a laser activates the fluorescent markers on the nucleotides as they go through the process. A sensor hooked up to a computer analyses the wavelength of the light and determines whether it represents an A, T, C, or G.
- The process must be repeated many times – for A, for T, C, and G. Repetitions make sure there are no errors. Fortunately, many copies of fragment 1 were made, so this is easy to check.
- Once fragment 1 is done, the lab technicians must process fragment 2, fragment 3, and so on, until all the fragments of the original sequence have been processed.

Thanks to modern communication technologies, it is possible for scientists working all over the world to collaborate and contribute to a scientific endeavour such as sequencing the genome of plants that help feed the world. Rice is one example: biologists from 10 countries contributed to sequencing the first rice genome.

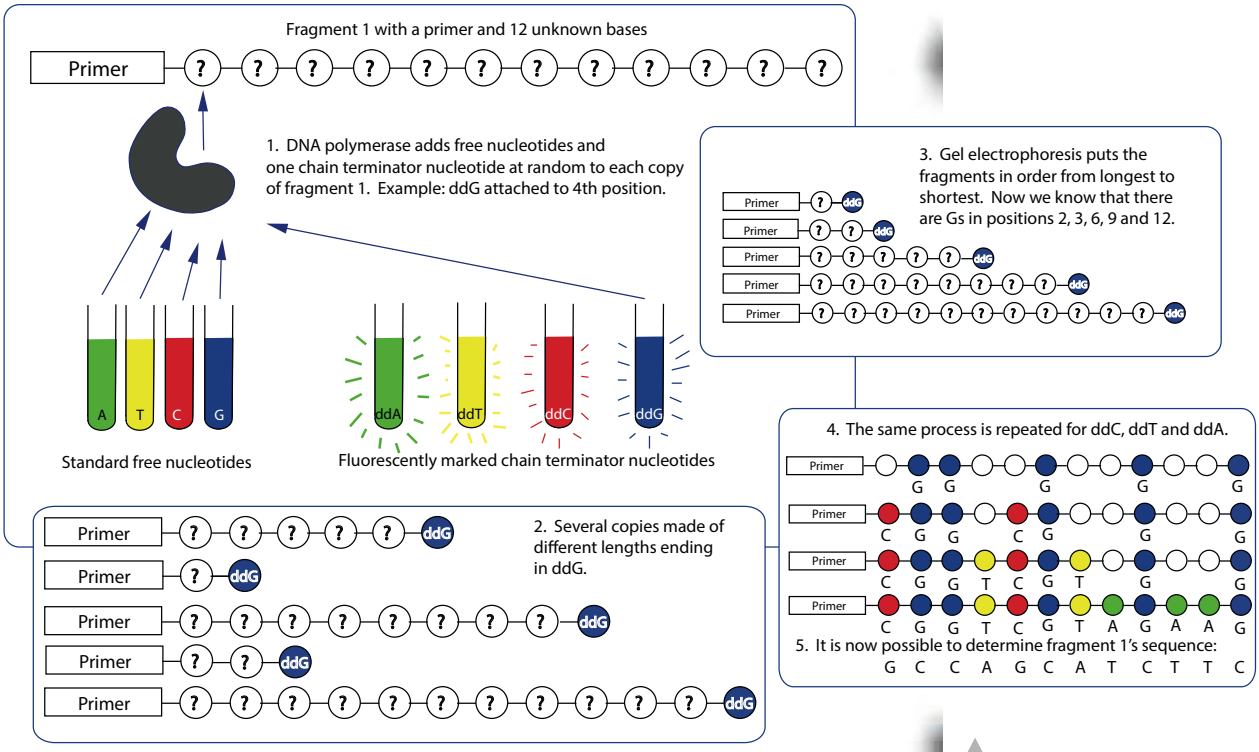


Figure 3.7 One method of DNA sequencing is called the Sanger technique.

- At this stage, the challenge is to put all the sequenced fragments of code together. When the original sequence was chopped up to make all these fragments, they became mixed up and out of order. Now that we know what their sequences are, we need to know the order in which to put them. This daunting task has been made easier by computers, but it consists of lining up any overlapping segments until they all match.

Since the Sanger technique was invented, many techniques have been developed to analyse each fragment only once, making it unnecessary to make multiple copies of each. This reduces the time and the cost of sequencing a genome. The objective of developing new sequencing techniques is to have a fast, inexpensive way to map anyone's genome.

The Human Genome Project

In 1990, an international cooperative venture called the Human Genome Project set out to sequence the complete human genome. Because the genome of an organism is a catalogue of all the bases it possesses, the Human Genome Project hoped to determine the order of all the bases A, T, C, and G in human DNA. In 2003, the Project announced that it had succeeded in achieving its goal. Now, scientists are working on deciphering which sequences represent genes and which genes do what. The human genome can be thought of as a map that can be used to show the locus of any gene on any one of the 23 pairs of chromosomes.

Before the Human Genome was mapped, fewer than 100 loci were known for genetic diseases. After the mapping was completed, more than 1400 were known, and today the number is in the thousands and increasing.

In the 1997 science fiction film *GATTACA*, one of the main characters brings a sample of cells to a walk-up window at an establishment that provides anonymous genome services. Within seconds, she gets a full printout and analysis of the genome of the man she wants to know more about. He is not aware that she is doing this. One objective of science fiction as an art form is to warn society of what might happen in the future if we are not careful. This film raises questions about how far technology will lead us and whether or not we want to go in that direction. Our society will need to make some difficult decisions in the coming years concerning our genomes and who has access to the information contained within them.

There is another international connection with the Human Genome Project in the sense that this project is a good example of scientists from all over the world working together. Dozens of nationalities participated in the project, and the results are available for free access worldwide thanks to online databases open to the public.

Delving into human genetics confirms two major themes:

- we are all the same
- we are all different.

On the one hand, the Human Genome Project has shown that there are only a very small number of DNA bases that make one person different from any other person in the world. This creates a feeling of unity, of oneness with all people. From peanut farmers in West Africa, to computer technicians in California, to fishermen in Norway, to businesswomen in Hong Kong, all humans carry inside them a common genetic heritage.

On the other hand, the Human Genome Project has shown that the small differences that do exist are important ones that give each person his or her uniqueness in terms of skin colour, facial features and resistance to disease, for example. These differences should be appreciated and celebrated as strengths. Unfortunately, they are often the basis of discrimination and misunderstanding.

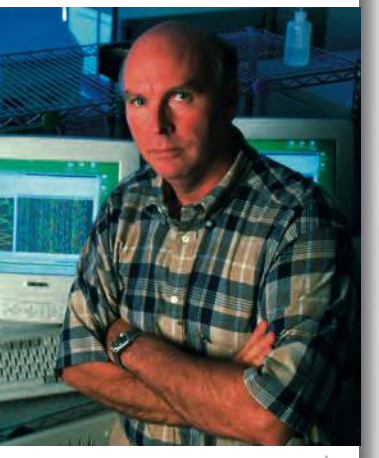
Can one genetic group be considered genetically superior to another? History has shown that many people think so, yet genetics shows that this is not the case. All human populations, whatever slight differences their genomes may have, deserve equal esteem as human beings.

Dr Francis Collins, one of the leaders of the Human Genome Project team.

In addition, by comparing the genetic makeup of populations around the world, countless details can be revealed about our ancestries and how human populations have migrated and mixed their genes with other populations over time. Without knowing it, you are carrying around in each one of your cells a library of information about your past.

TOK

Many companies offer genome sequencing for private citizens willing to pay the price. Some of the products offered are revelations about ancient family origins and risk factors for some health problems, such as the chances of developing certain types of cancer or heart disease. Would you want to know if there was a chance that your life could be suddenly shortened by the presence or absence of a certain gene? Would you tell your family and friends? Would you want your parents to do such a test? Should people tell their employer or each other about any health-related issues revealed by a genomic analysis? Or, on the contrary, is this a private, personal thing that no one else needs to know about? How accurate and reliable are these analyses? Should we believe everything they say?



Dr Craig Venter, one of the leaders of the Human Genome Project team.

Using DNA to make medicines

Another advantageous use of the human genome is the production of new medications. This process involves several steps:

- find beneficial molecules that are produced naturally in healthy people
- find out which gene controls the synthesis of a desirable molecule
- copy that gene and use it as instructions to synthesize the molecule in a laboratory
- distribute the beneficial therapeutic protein as a new medical treatment.

This is not science fiction: genetic engineering firms are finding such genes regularly. One current line of research is dealing with genes that control ageing. How much money do you think people would be willing to pay for a molecule that could reverse the effects of ageing and prolong life by several decades?

What if a biotech company finds a useful human gene in your body? For example, a gene that produces a protein to help balance cholesterol levels in the body and prevent heart problems. Can the company patent that gene in order to protect its discovery and in order to earn money from it? With a patent, the company could charge pharmaceutical manufacturers that wanted to use the gene to make new medicines. In many countries there are few if any laws about such things because the techniques are so new.

CHALLENGE YOURSELF

3 Cytochrome *c* is a protein found in mitochondria and it plays a key role in cell respiration by shuttling electrons from one place to another. If an organism did not have the genetic code to make cytochrome *c*, it could not survive. By comparing the genetic sequence used to produce this protein in various species, scientists were able to see how mutations accumulate over time. The differences between a horse's base sequence for this protein and a zebra's is much smaller than the differences between a horse's and a lizard's. Humans and chimpanzees have identical cytochrome *c* amino acid sequences, whereas the yeast *Candida krusei* has 51 amino acid differences compared with humans.

For this exercise, find the relevant PDF file in the hotlinks at the end of this section. Follow the instructions to compare the genetic sequences for various organisms for the gene that makes cytochrome *c*.

Use the hotlinks to find ancient sequences of base pairs to compare between species of prokaryotes. Can you find a correlation between the numbers of mutations and the evolutionary distance between species?

NATURE OF SCIENCE

Why are scientists interested in comparing the genetic codes of various species? For one thing, when looking at a gene that every living thing should have, such as a gene for how to make ribosomes, the number of mutations a species has in that gene compared with another species gives insight into how closely they are related to each other.

Because of a certain number of differences in metabolism and genetic makeup in types of single-celled organisms that looked similar to bacteria, the biologist Carl Woese proposed the domain Archaea to distinguish them from bacteria (prokaryotes) and eukaryotes. Although Archaea do not have a nucleus, they have enough differences compared with prokaryotes to set them apart from other bacteria. Among the species in this group are single-celled organisms that thrive in very salty conditions, some that live in hot springs at extreme temperatures, and many others that live in the soil or in the ocean: some might be living in you or on you right now.

It took decades for Woese's proposal to be accepted, but the overwhelming evidence in Archaea's favour made it very difficult for opponents of the idea to argue against it.

Can human genes be patented?

In the spring of 2013, the United States Supreme Court heard a landmark case between a biotech company, Myriad Genetics (the defendant), and the Association for Molecular Pathology, AMP (the plaintiff), a group of genetics experts who specialize in many things, including the diagnosis of genetic diseases and disorders. Myriad had a patent on naturally occurring human genes called *BRCA*, which can be used to tell whether a woman has a genetically increased chance of breast cancer or ovarian cancer, two of the most common and deadly cancers in Western society today. AMP was taking Myriad to court because they thought the *BRCA* gene sequences should be available freely for diagnosing cancer. AMP thought that it was unfair that clinical

A patent is an authorization for a person or a company to make, use, or sell an invention, and it makes it illegal for anyone else to make, use, or sell it. For example, Thomas Edison had more than 1000 patents for the many things he invented, such as his improved electric light bulb and his phonograph. In the medical field, it is common to patent new pharmaceutical molecules developed in laboratories so that only the initial company that invented the drug can manufacture and sell it. Typically, a patent filed today is limited to 20 years.

teams could not access the *BRCA* genes to do their own testing and diagnosis. They argued that a company such as Myriad should not be able to put an industrial patent on genes, because DNA sequences occur naturally and are not invented by a company: therefore, they are not patentable objects.

Myriad's argument was that, although DNA is found in nature, genes are all connected to each other, whereas the isolated sequences for which they had patents could only be the product of a biotech laboratory using sophisticated equipment to do the separation and identification: therefore, the DNA sequences in question were not in their natural form. The researchers at Myriad were the first to patent these fragments of DNA and recognize their usefulness. They patented their *BRCA* genes just as any pharmaceutical company would patent a new molecule that they thought would make a useful medicine. These patents, and the diagnostic tests associated with them, have made Myriad a very successful and profitable company. Because it is their intellectual property, anyone who wants a genetic test for breast cancer or ovarian cancer must go to them. Myriad argued that taking away their patents would take away their livelihood because it would allow any company to develop and perform their own diagnostic tests.

Biotechnology is posing a rising number of challenges to the legal institutions of the world.

Another argument from the plaintiff was that, because Myriad was the sole company to administer *BRCA* diagnostics, it was impossible for a patient to get a second opinion, a key step in the diagnosis and treatment of a medical condition as serious as cancer. Also, Myriad could charge high fees because they had no competition in the market. Myriad's justification for the cost of the tests was that biotech research requires very expensive laboratory equipment and highly trained professionals, so the money earned from the diagnostic tests helps the company invest in new developments to advance scientific knowledge and continue putting new diagnostic tools in place.

In the end, the US Supreme Court found it unconstitutional to patent a DNA sequence found in nature. Justice Clarence Thomas wrote 'A naturally occurring DNA segment is a product of nature and not patent eligible merely because it has been isolated.'

To learn more about genes, go to the hotlinks site, search for the title or ISBN, and click on Chapter 3: Section 3.1.

Exercises

1. What is the difference between an allele and a gene?
2. Give an example of a mutation in an eagle's offspring that could be considered a beneficial mutation.
3. Explain why eukaryotic chromosomes always come in pairs.

3.2 Chromosomes

Understandings:

- Prokaryotes have one chromosome consisting of a circular DNA molecule.
- Some prokaryotes also have plasmids but eukaryotes do not.
- Eukaryote chromosomes are linear DNA molecules associated with histone proteins.
- In a eukaryote species there are different chromosomes that carry different genes.
- Homologous chromosomes carry the same sequence of genes but not necessarily the same alleles of those genes.
- Diploid nuclei have pairs of homologous chromosomes.
- Haploid nuclei have one chromosome of each pair.
- The number of chromosomes is a characteristic feature of members of a species.
- A karyogram shows the chromosomes of an organism in homologous pairs of decreasing length.
- Sex is determined by sex chromosomes and autosomes are chromosomes that do not determine sex.

Applications and skills:

- Application: Cairns' technique for measuring the length of DNA molecules by autoradiography.
- Application: Comparison of genome size in T2 phage, *Escherichia coli*, *Drosophila melanogaster*, *Homo sapiens*, and *Paris japonica*.
- Application: Comparison of diploid chromosome numbers of *Homo sapiens*, *Pan troglodytes*, *Canis familiaris*, *Oryza sativa*, and *Parascaris equorum*.
- Application: Use of karyograms to deduce sex and diagnose Down syndrome in humans.
- Skill: Use of databases to identify the locus of a human gene and its polypeptide product

Guidance

- *The terms karyotype and karyogram have different meanings. Karyotype is a property of a cell: the number and type of chromosomes present in the nucleus, not a photograph or diagram of them.*
- *Genome size is the total length of DNA in an organism. The examples of genome and chromosome number have been selected to allow points of interest to be raised.*
- *The two DNA molecules formed by DNA replication prior to cell division are considered to be sister chromatids until the splitting of the centromere at the start of anaphase. After this, they are individual chromosomes.*

NATURE OF SCIENCE

Developments in research follow improvements in techniques: autoradiography was used to establish the length of DNA molecules in chromosomes.

The chromosome in prokaryotes

You will recall from Chapter 1 that the nucleoid region of a bacterial cell contains a single, long, continuous, circular thread of DNA. Therefore, this region is involved with cell control and reproduction.

Notice how the presence of a single circular chromosome is a very different situation from all the cells we looked at in Section 3.1, which always had chromosomes in pairs. Why is this? Prokaryotes can reproduce using binary fission (dividing), whereas organisms such as plants and animals more frequently use sexual reproduction (involving a male and a female). Any time two parents are involved, the offspring will have pairs of chromosomes rather than single chromosomes. Because prokaryotes have only one parent, they have only one chromosome.

Some prokaryotes also have plasmids but eukaryotes do not

Escherichia coli, like many prokaryotes (bacteria), have small loops of DNA that are extra copies of some of the genetic material of the organism. These loops are called

plasmids. These small, circular, DNA molecules are not connected to the main bacterial chromosome. The plasmids replicate independently of the chromosomal DNA. Plasmid DNA is not required by the cell under normal conditions, but it may help the cell adapt to unusual circumstances. Plasmids can also be found in Archaea as well as in bacteria.

As we will see later in Section 3.5, these loops can be used in genetic engineering. Genetic manipulation using plasmids is not possible in eukaryotes such as plants and animals, because they do not have plasmids. Other techniques must be used for genetically modified (GM) crops and animals, which we will discuss later (also in Section 3.5).

Eukaryote chromosomes

The DNA of eukaryotic cells most often occurs in the form of chromosomes. Chromosomes carry information necessary for the cell to exist. This allows the organism, whether unicellular or multicellular, to survive. DNA is the genetic material of the cell. It enables certain traits to be passed on to the next generation. When the cell is not dividing, the chromosomes are not visible structures. During this phase, the cell's DNA is in the form of chromatin. Chromatin is formed of strands of DNA and proteins called histones.

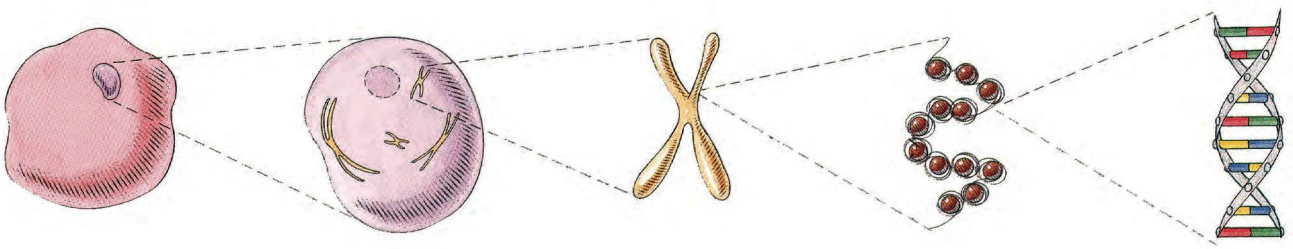


Figure 3.8 This drawing shows how DNA is packaged into chromosomes.

When looking at unfolded DNA with an electron microscope, you can see what looks like beads on a string. Each of the beads is a nucleosome. A nucleosome consists of two molecules of each of four different histones. The DNA wraps twice around these eight protein molecules. The DNA is attracted to the histones because DNA is negatively charged and the histones are positively charged. Between the nucleosomes is a single string of DNA. There is often a fifth type of histone attached to the linking string of DNA near each nucleosome. This fifth histone leads to further wrapping (packaging) of the DNA molecule and eventually to the highly condensed or supercoiled chromosomes.

When DNA is wrapped around the histones and then further wrapped in even more elaborate structures, it is inaccessible to transcription enzymes. Therefore, the wrapping or packaging of DNA regulates the transcription process. This allows only certain areas of the DNA molecule to be involved in protein synthesis.

Multiple chromosomes

As shown in Table 3.2, eukaryotes have more than one chromosome. Most eukaryotes have multiple pairs of chromosomes, and each chromosome will carry a different set of instructions for the cell.

Table 3.2 A comparison of eukaryote chromosomes and prokaryote chromosomes

	Prokaryote	Eukaryote
Number of chromosomes	1	2 or more*
Shape	Circular	Linear
Histones	Not present**	Present
Presence of plasmids	Sometimes	Never
Organized into pairs	No	Yes

*It is rare for eukaryotes to have one chromosome, but some can, such as male bees, wasps, and ants.
**Among prokaryotes, archaeans have the same properties as bacteria (prokaryotes) in this table with the exception that histones are present in archaen DNA but not in bacterial DNA.

Homologous chromosomes: the same genes but not always the same alleles

In a typical human cell, the 46 chromosomes can be grouped into 23 pairs of chromosomes called homologous chromosomes. Homologous means similar in shape and size, and it means that the two chromosomes carry the same genes. The example in Figure 3.9 shows one of the 23 pairs of homologous chromosomes found in humans.

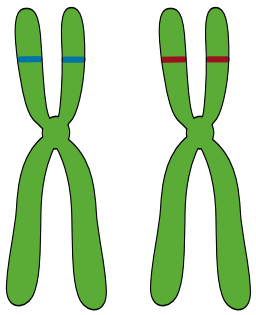


Figure 3.9 Homologous chromosomes. Although these are the same size and shape, and carry the same genes, the different coloured bands on the short arms of each chromosome reveal that they do not carry the same allele of the gene at the locus shown.

Remember that the reason there are two of each chromosome is that one came from the father and the other from the mother. Although a pair of homologous chromosomes carries the same genes, they are not identical because the alleles for the genes from each parent could be different. In Figure 3.9, we can see that the locus shown contains different coloured bands, revealing that this individual got a different allele from his or her mother than from his or her father for this particular gene.

It is important to note that the shapes you see in Figure 3.9 represent two chromosomes together as a single pair, but that each chromosome has been doubled as a result of DNA replication. Chromosomes only look like this when the cell they are in is getting ready to divide. At this stage, the two blue-banded zones are part of two connected sister chromatids forming a single chromosome attached at the centromere. Likewise, the two red-banded zones belong to two sister chromatids. Each chromatid includes the long arm as well as the short arm (the one that contains the coloured bands in this example). This will be important to remember later, when we watch the sister chromatids split during cell division. When the chromatids separate, they become two identical chromosomes. But as long as they are attached at the centromere, they are considered to be part of a single chromosome.

Examining chromosomes in root tips

Safety alerts: The chemicals in this lab, as well as the risk of breaking glass during the squashing process, require vigilance and caution. Ask your teacher what precautions to consider.

There are two options for doing this lab, depending on time and materials available. You can either prepare your own root tip squashes from plant material grown in the laboratory, or you can examine pre-made root tip preparations from a laboratory supply company.

For the first option, carry out the following.

- Over a beaker full of water, suspend a plant that will produce roots in the water, for example garlic, onion, or potato. Use toothpicks to support it.
- Leave it for 2–5 days until little white roots have pushed their way down into the water. Top up the water periodically if it gets low.
- Cut off the roots and place them first into ethanoic acid for 10 min, then into 1 M HCl for 10 min, then rinse them with water.
- Cut off 2 mm of the tips, and place these segments on a microscope slide.
- Stain them with orcein, allowing it to soak in for a few minutes.
- To spread the cells out on the slide, use a mounted needle.
- Place a cover slip over the root tips, and place several layers of paper towel over the slide and cover slip. Push down firmly to squash the tissue.
- If you have the time and materials, you can compare the chromosomes in your root tips with professionally prepared slides.

Diploid and haploid cells

The term diploid is used to describe a nucleus that has chromosomes organized into pairs of homologous chromosomes. Most cells in the human body are diploid cells, and in such cells the nucleus contains a set of 23 chromosomes from the mother and 23 from the father. There is a category of cells that only contain 23 chromosomes in total: the sex cells, also called gametes. Because the chromosomes in sperm and egg cells do not come in pairs, but rather only have a single chromosome from each pair, they are said to be haploid. The adult form of animal cells is rarely haploid, but there are exceptions, for example male bee, wasp, and ant cells are haploid. Generally speaking, the vast majority of cells in sexually reproducing organisms are diploid, and only the gametes are haploid.

The variable n represents the haploid number, and it refers to the number of sets of chromosomes that a nucleus can have. For a human egg cell, $n = 23$. When an egg cell is fertilized by a sperm cell (a sperm is also haploid and therefore contains 23 chromosomes), a zygote is formed and the two haploid nuclei fuse together, matching up their chromosomes into pairs. Hence humans generally have a total of 23 + 23 = 46 chromosomes. This means that in humans, $2n = 46$, so diploid cells in humans have 23 pairs of chromosomes making a total of 46 chromosomes. Compare this number with some of the other species in Table 3.3.

Table 3.3 A comparison of types of cells and chromosome numbers

	Types of cells and chromosome numbers	
Species	Haploid = *n*	Diploid = 2*n*
Human (*Homo sapiens*)	23	46
Chimpanzee (*Pan troglodytes*)	24	48
Domestic dog (*Canis familiaris*)	39	78
Rice (*Oryza sativa*)	12	24
Roundworm (*Parascaris equorum*)	1	2

Chromosome number: a defining feature

As you can see, the number 46 for humans is very different compared with the number for a worm. One of the best-studied worms in genetics laboratories is *Caenorhabditis elegans*, whose genome was first sequenced in 1998. It has six chromosomes, meaning its diploid number, 2*n*, is 6, and therefore its haploid number, *n*, is 3. It would be expected that all the cells in *C. elegans* would have six chromosomes, and, likewise, that all cells in humans would have 46. Although this is true for most cells, we have already seen the exception of haploid cells (*n*), and we will see later that some people can be born with chromosomes missing (45 or fewer) or with extra chromosomes (47 or greater), but these remain exceptions. In addition, some cells do not contain a nucleus and have no chromosomes to show, such as red blood cells. Generally speaking, however, the number of chromosomes is a characteristic feature of the cells of a species.

Karyograms and karyotypes

A karyogram is a representation of the chromosomes found in a cell arranged according to a standard format, as in the example in the photo opposite. The chromosomes are placed in order according to their size and shape. The shape depends mainly on the position of the centromere. A karyogram is used to show a person's karyotype, which is the specific number and appearance of the chromosomes in his or her cells.

How is such an image obtained? Once the cells of an organism have been collected and grown in culture, a karyogram is made following the steps below. For an explanation of how the cells are collected, see Section 3.3.

1. The cells are stained and prepared on a glass slide, to see their chromosomes under a light microscope.
2. Photomicrograph images are obtained of the chromosomes during a specific phase of cell division called the mitotic metaphase (see Section 1.6).

CHALLENGE YOURSELF

4 Use the karyogram in the photo below to determine whether the child is a boy or a girl. How do you know? Does the child's karyotype include any anomalies? If so, describe what you see.

This is a karyogram showing all 23 pairs of chromosomes. What can we learn about the individual's karyotype from this figure? This karyogram was prepared using false colour imagery.

3 The images are cut out and separated, a process that can be done using scissors or using a computer.

4 The images of each pair of chromosomes are placed in order by size and the position of their centromeres. Generally speaking, the chromosomes are arranged in order by decreasing length. The exception is in the 23rd pair of chromosomes, which can contain one or two X chromosomes, which are considerably larger than the chromosomes in the 22nd pair (see the chromosome pair marked X in the photo).

Sex determination

The 23rd pair of chromosomes are called the sex chromosomes because they determine whether a person is a male or a female. The X chromosome is longer than the Y chromosome, and contains many more genes. Unlike the other 22 pairs of chromosomes, this is the only pair in which it is possible to find two chromosomes that are very different in size and shape.

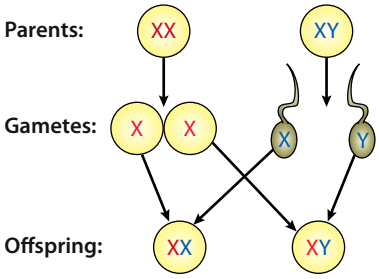


Figure 3.10 How sex is determined: will the baby be a boy or a girl?

In human females there are two X chromosomes. When women produce gametes, each egg will contain one X chromosome. Human males have one X chromosome and one Y chromosome. When males produce sperm cells, half of them contain one X chromosome and half contain one Y chromosome. As a result, when an egg cell meets a sperm cell during fertilization, there is always a 50% chance that the child will be a boy and a 50% chance that the child will be a girl (see Figure 3.10):

- XX = female
- XY = male.

The chances remain the same no matter how many boys or girls the family already has.

Any chromosome that is not a sex chromosome is called an autosome, or autosomal chromosome. Humans have 22 pairs of autosomes and one pair of sex chromosomes (see Figure 3.11).

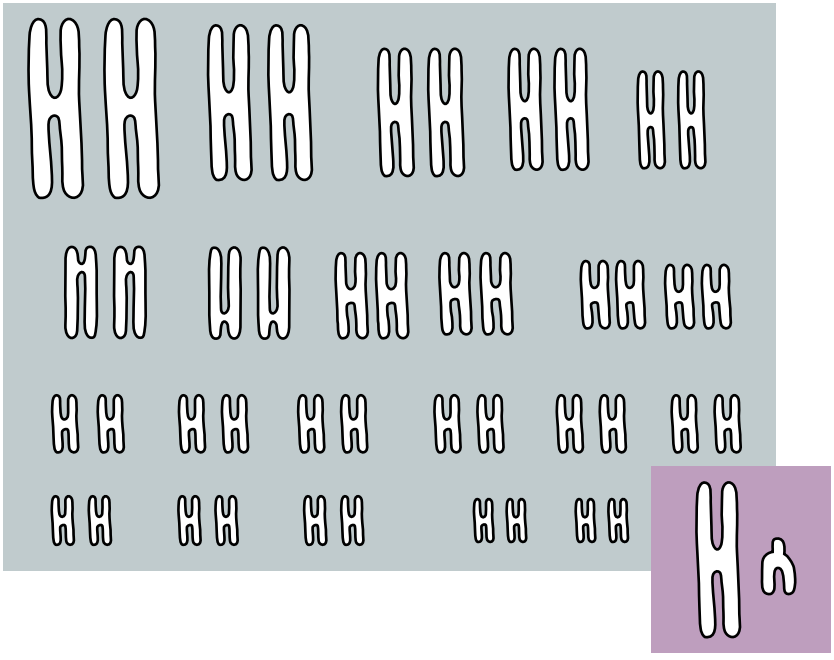


Figure 3.11 Human chromosomes: grey = autosomes, purple = sex chromosomes.

If a trait or gene is described as autosomal, its locus is on one of the 22 pairs of autosomes, not on the sex chromosomes. Where a gene is located determines whether or not the trait it controls is more common in males or females. When a trait is more common in one sex than the other, there is a good chance that the trait is sex-linked, and that the locus of the gene is on either the X chromosome or the Y chromosome (see sex linkage in Section 3.4). If there is no pattern to the frequency of a trait between females and males, it is most likely to be an autosomal trait.

Autoradiography

Autoradiography is a technique in which radiation from a substance is captured on photographic film or by a camera sensor. Unlike an X-ray, during which the film or sensor is exposed to an external source of radioactivity, autoradiograms (the images formed by autoradiography) are exposed to radioactive particles being given off by the substance itself. This technique has been described as structures such as DNA being able to 'take their own pictures'. It is used in genetics work to obtain images of DNA strands so that their lengths can be measured.

Cairns' technique involves injecting radioactive materials into the DNA samples that will expose the film faster. Such materials are called radio markers. In the case of measuring the lengths of DNA strands, the DNA forming during replication is given a radioactive form of a molecule called thymidine. Thymidine is a component of a DNA nucleotide made up of a pentose sugar bonded to thymine; it is represented by the letter T in the genetic code. The radioactive form added in the experiment is called ^{3}H-thymidine, in which the ^{3}H is the radioactive isotope of hydrogen. An isotope is a version of an atom with a different atomic mass compared with other versions of the same atom, usually because it has more neutrons. The radioactive ^{3}H molecule is used as a radio marker to keep track of where those thymidine molecules are, because it leaves traces of its presence on photographic film.

This technique was used by John Cairns in 1962 to demonstrate that a bacterium's chromosome is made up of a single circle of DNA and that it is replicated by being unzipped. The photos he took using autoradiography looked like the image below, and Cairns called them theta structures because they were reminiscent of the Greek letter theta (θ).

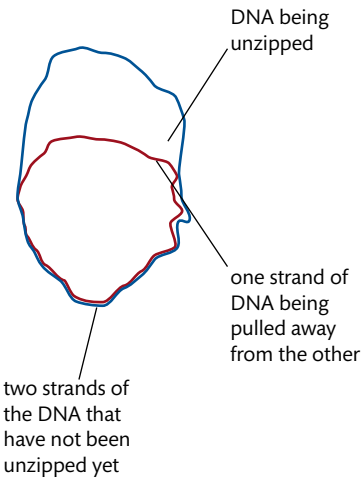


Figure 3.12 A diagram of what Cairns saw when the circular DNA chromosome of *E. coli* was being unzipped for replication.

CHALLENGE YOURSELF

5 Scientists have long dreamed of the moment when they can see the mysterious secret code of life. For example, discovering the code behind something as wonderfully useful as insulin, the protein in your blood that helps regulate blood sugar, was the dream for many decades. Wouldn't it be nice to be able to read the code, copy it, and use the copy to make insulin in a laboratory? In the early 1980s, a small company called Genentech was the first to make laboratory-synthesized insulin available to patients who needed it to treat their diabetes. The company went on to many other projects in the field of biotechnology and, three decades later, the company was worth tens of billions of dollars.

Today, a lot of the discoveries that took months or years to make are just a few clicks away, because they are available for everyone to consult. The online genetic database at the National Center for Biotechnology Information (NCBI), for example, has many genes that you yourself can look up. Interested in insulin or haemoglobin? Search for those words or their genes (*INS* for insulin or *HBB* for one of the subunits of haemoglobin). At the end of this section, use the hotlinks to see if you can find out at what position and on what chromosome you can find the secret code for these valuable molecules of life. If you ask for the FASTA data (pronounced 'Fast A'), you can see every A, T, C, and G that makes up a gene coding for a protein. Also, check out the NCBI 1000 Genome Browser, an online map of human genes chromosome by chromosome. If you get lost, they have video tutorials to help.

To learn more about chromosomes, go to the hotlinks site, search for the title or ISBN, and click on Chapter 3: Section 3.2.

Exercises

4 Draw and label a chromosome. Include the following labels: chromatid, centromere. Indicate an example of a locus.

5 Explain why prokaryotes are never diploid.

NATURE OF SCIENCE

Making careful observations: meiosis was discovered by microscope examination of dividing germ-line cells.

3.3 Meiosis

Understandings:

- One diploid nucleus divides by meiosis to produce four haploid nuclei.
- The halving of the chromosome number allows a sexual life cycle with fusion of gametes.
- DNA is replicated before meiosis so that all chromosomes consist of two sister chromatids.
- The early stages of meiosis involve pairing of homologous chromosomes and crossing over followed by condensation.
- Orientation of pairs of homologous chromosomes prior to separation is random.
- Separation of pairs of homologous chromosomes in the first division of meiosis halves the chromosome number.
- Crossing over and random orientation promotes genetic variation.
- Fusion of gametes from different parents promotes genetic variation.

Applications and skills:

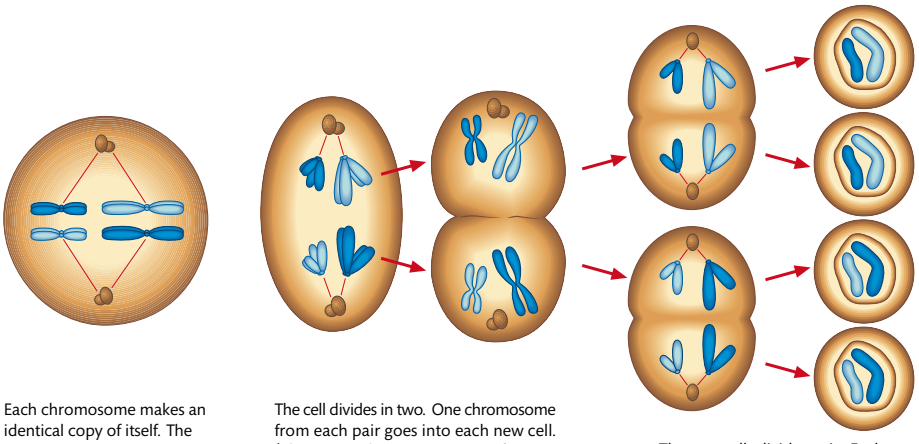
- Application: Non-disjunction can cause Down syndrome and other chromosome abnormalities.
- Application: Studies showing age of parents influences chances of non-disjunction.
- Application: Description of methods used to obtain cells for karyotype analysis, e.g. chorionic villus sampling and amniocentesis, and the associated risks.
- Skill: Drawing diagrams to show the stages of meiosis resulting in the formation of four haploid cells.

Guidance

- *Preparation of microscope slides showing meiosis is challenging and permanent slides should be available in case no cells in meiosis are visible in temporary mounts.*
- *Drawings of the stages of meiosis do not need to include chiasmata.*
- *The process of chiasmata formation need not be explained.*

Producing four haploid nuclei

The vast majority of cells in a person's body each contains 46 chromosomes. Gametes (sperm cells and egg cells) cannot contain 46 chromosomes for the simple reason that, if they did, when they fused together during fertilization, the baby that would be formed would have a total of 92 chromosomes, and each new generation would double its chromosome number, making an impossibly large amount of DNA to deal with. To avoid this problem of accumulating too many chromosomes, humans and other animals produce egg cells and sperm cells in such a way that the number of chromosomes in their nuclei is halved. Hence, sperms and eggs only contain 23 chromosomes, one from each pair, rather than complete pairs. In order to make such special cells with half the chromosomes, a special type of cell division is needed: meiosis. Such a splitting is called a reduction division.



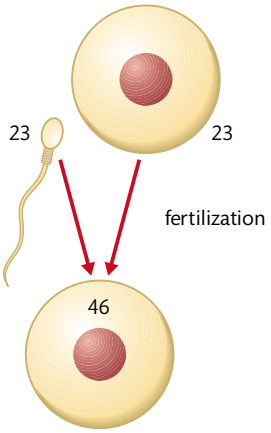
Each chromosome makes an identical copy of itself. The two copies stay in contact with each other.
The chromosomes line up in pairs. Each pair codes for the same characteristics.

The cell divides in two. One chromosome from each pair goes into each new cell. (This means there are no pairs of chromosomes in the new cells.)

The new cells divide again. Each chromosome splits in two. Each half goes into a new cell.
This makes four haploid cells. These develop into gametes.

Figure 3.13 How the chromosome number is halved. More details about the specific stages of meiosis appear later in this chapter.

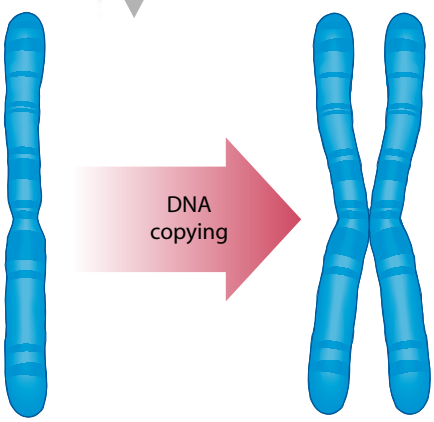
Figure 3.14 How chromosome number is maintained in the sexual life cycle.



The halving of the chromosome number

Whereas mitosis produces diploid ($2n$) nuclei containing 46 chromosomes (organized into 23 pairs), meiosis produces haploid (n) nuclei that contain 23 chromosomes, each representing half of one pair. Notice in Figure 3.13, from a single cell on the left, four cells were produced on the right. Notice also that the number of chromosomes in the example is 4 in the parent cell at the start (so $2n = 4$), because there are 2 in each pair. In contrast, the number of chromosomes at the end is only 2 ($n = 2$), because each 'pair' is not a pair anymore but rather a single representative from each pair. In the testes and ovaries, respectively, meiosis produces haploid sperms and eggs, so that, when fertilization occurs, the zygote will receive 23 + 23 = 46 chromosomes; half from the mother, and half from the father. This is how the problem of changing chromosome number is avoided. As a result, the human number of 46 is preserved by the sexual life cycle.

Figure 3.15 An artist's conception of a single chromosome before and after DNA replication.



DNA is replicated before meiosis

The reason why chromosomes are represented as having the shape reminiscent of the letter 'X' or 'H', as used in the previous section, is because

TOK

Sometimes it takes a while before an established scientific idea can be modified. It was decided in 1922 that the number of chromosomes in humans was 48 (24 pairs). This number is in line with other ape species that are the closest species to humans, genetically speaking. We know now that the number is 46 (23 pairs), and that number was accepted in the 1950s. Photographs of human cells from long before the 1950s clearly show 46 chromosomes. What does this reveal about established knowledge in science? Why do you think it takes so many years to change an established idea?

at this stage in the chromosome's existence, the DNA has been replicated so that a full copy of the original DNA has been produced.

As a result, the single chromosome comprises two sister chromatids side-by-side and joined in the middle at the centromere (see Figure 3.15).

In reality, before the chromosomes start preparing for the cell to divide, they are all uncoiled and are not visible in the nucleus. This is one of the reasons why, when looking at cells under a microscope, it is not usually possible to see chromosomes all coiled up. It is only in the early stages of the preparation for cell division that condensation happens and the chromosomes coil up into the shapes you are being shown in this chapter.

Pairing of homologous chromosomes and crossing over

Meiosis is a step-by-step process by which a diploid parent cell produces four haploid daughter cells. Before the steps begin, DNA replication allows the cell to make a complete copy of its genetic information during interphase. This results in each chromatid having an identical copy, or sister chromatid, attached to it at the centromere.

In order to produce a total of four cells, the parent cell must divide twice: the first meiotic division makes two cells, and then each of these divides during the second meiotic division to make a total of four cells.

One of the characteristics that distinguishes meiosis from mitosis (see Section 1.6) is that, during the first step, called prophase I, there is an exchange of genetic material between non-sister chromatids in a process called crossing over (see Figure 3.16). This trading of segments of genes happens when sections of two homologous chromatids break at the same point, twist around each other, and then each connects to the other's initial position.

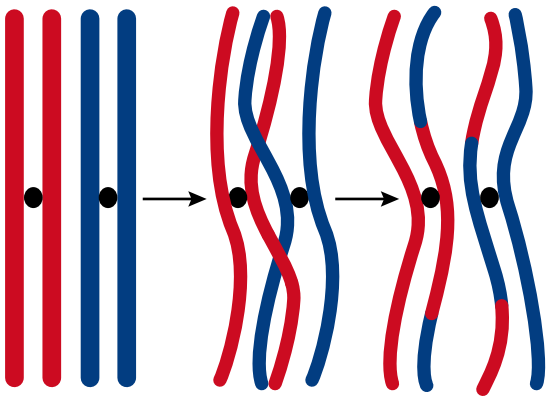


Figure 3.16 Crossing over occurring in a pair of homologous chromosomes.

Crossing over allows DNA from a person's maternal chromosomes to mix with DNA from the paternal chromosomes. In this way, the recombinant chromatids that end up in the sperm or the egg cells are a mosaic of the two parent cells' original chromatids. This helps increase the variety among offspring from the same two parents, and so increases the chances of survival of some offspring if one combination of alleles is more favourable for survival than others.

Meiosis I takes place in order to produce two cells, each with a single set of chromosomes (see Figure 3.17).

Random orientation

Figure 3.17 shows that, during metaphase I, the homolgous pairs of chromosomes line up along the centre of the cell. The way that they happen to line up is by chance, and that is why it is called random orientation. As seen with crossing over, this is another adaptation that increases variety in the offspring. The result of random orientation is that a male will only very rarely produce two sperm cells that are identical. Likewise, for a female, it is highly likely that she will never produce the same egg twice in her lifetime. These are among the reasons why a couple will never have the same offspring twice. The only way that a male and a female can naturally have the same offspring twice is by producing identical twins, but, in this case, it is two children from the same egg cell and the same sperm cell.

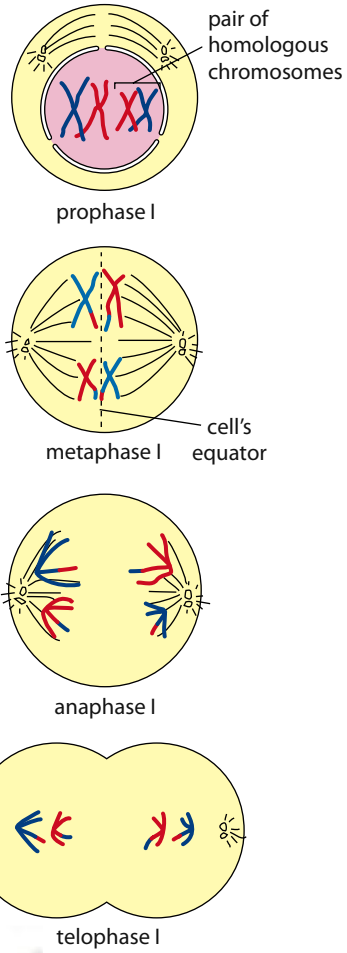


Figure 3.17 The stages of meiosis I.

Halving the chromosome number

Prophase I

1. Chromosomes become visible as the DNA becomes more compact.
2. Homologous chromosomes, also called homologues, are attracted to each other and pair up: one is from the individual's father, the other from the mother.
3. Crossing over occurs.
4. Spindle fibres made from microtubules form.

Metaphase I

1. The homologous chromosomes line up across the cell's equator by random orientation.
2. The nuclear membrane disintegrates.

Anaphase I

Spindle fibres from the poles attach to chromosomes and pull them to opposite poles of the cell.

Telophase I

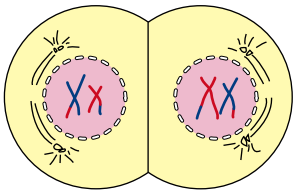
1. Spindles and spindle fibres disintegrate.
2. Usually, the chromosomes uncoil and new nuclear membranes form.
3. Many plants do not have a telophase I stage.

At the end of meiosis I, cytokinesis happens: the cell splits into two separate cells. The cells at this point are haploid because they contain only one chromosome of each pair. However, each chromatid still has its sister chromatid attached to it, so no S phase is necessary.

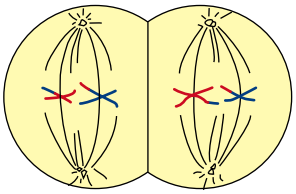
Now meiosis II takes place in order to separate the sister chromatids (see Figure 3.18).

Prophase II

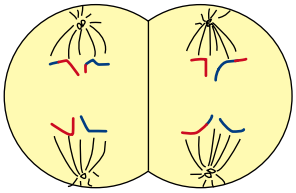
1. DNA condenses into visible chromosomes again.
2. New meiotic spindle fibres are produced.



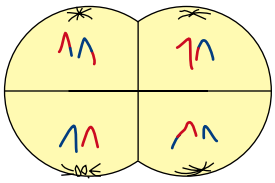
prophase II



metaphase II



anaphase II



telophase II

Figure 3.18 Meiosis II.

Metaphase II

1 Nuclear membranes disintegrate.

2 The individual chromosomes line up along the equator of each cell in no special order; this is called random orientation.

3 Spindle fibres from opposite poles attach to each of the sister chromatids at the centromeres.

Anaphase II

1 Centromeres of each chromosome split, releasing each sister chromatid as an individual chromosome.

2 The spindle fibres pull individual chromatids to opposite ends of the cell.

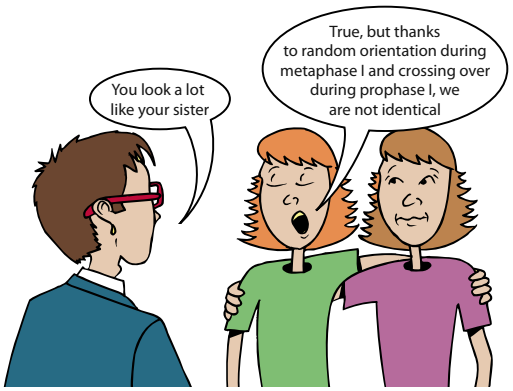
3 Because of random orientation, the chromatids could be pulled towards either of the newly forming daughter cells.

4 In animal cells, cell membranes pinch off in the middle, whereas in plant cells new cell plates form to demarcate the four cells.

Telophase II

1 Chromosomes unwind their strands of DNA.

2 Nuclear envelopes form around each of the four haploid cells, preparing them for cytokinesis.



Fertilization and variation

As can be seen with siblings from the same mother and father who are not identical twins, crossing over during prophase I and random orientation during metaphase I allow variation in the offspring. There is one other way that genetic variation is also promoted: fertilization. When the egg and sperm cells meet, there is a great deal of chance involved. For example, a man can produce millions of different sperm cells, each with a unique combination of half his DNA.

How is this calculated? If only the number of chromosomes in each haploid cell (n) is considered, the calculation is 2^n because there are two possible chromosomes in each pair (maternal and paternal) and there are n chromosomes in all. For humans, the number is 2^{23} because there are 23 chromosomes in each gamete. So the probability that a woman could produce the same egg twice is 1 in 2^{23} or 1 in 8 388 608. Even this calculation is an oversimplification, however, because it does not take into consideration the additional variety that results from crossing over.

In addition, the calculation 2^n only considers one gamete. To produce offspring, two gametes are needed, and the chances that both parents produce two identical offspring (apart from identical twins) is infinitesimal.

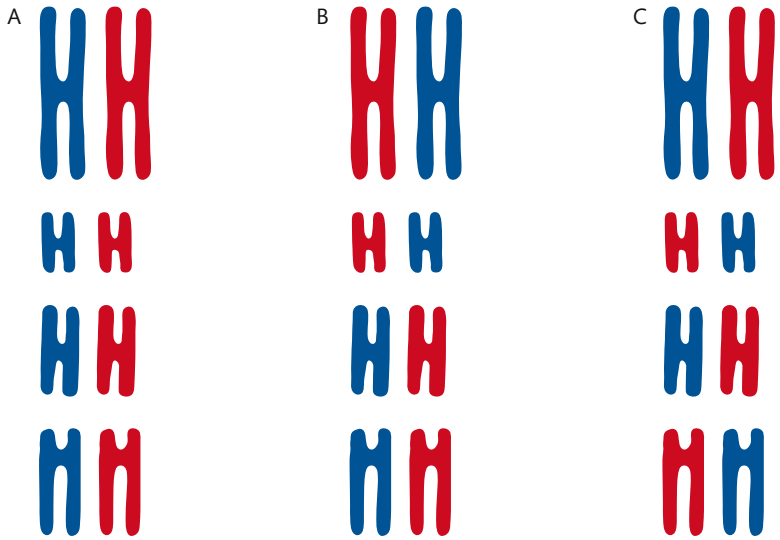


Figure 3.19 Rows A, B, and C show three of the sixteen possible orientations for four pairs of homologous chromosomes. In humans there are 23 pairs with more than 8 million possible orientations.

Extra or missing chromosomes

Sometimes errors occur during meiosis and a child can receive an atypical number of chromosomes, such as 47 instead of 46. One such anomaly is called Down syndrome, and it happens when there is an extra chromosome in the 21st pair. The extra chromosome results from a phenomenon called non-disjunction, which can happen at different times but most often occurs when the 21st pair of homologous chromosomes fails to separate during anaphase I. Hence, the egg the woman produces has two 21st chromosomes instead of one. And when a sperm cell fertilizes the egg, the total number of 21st chromosomes is three.

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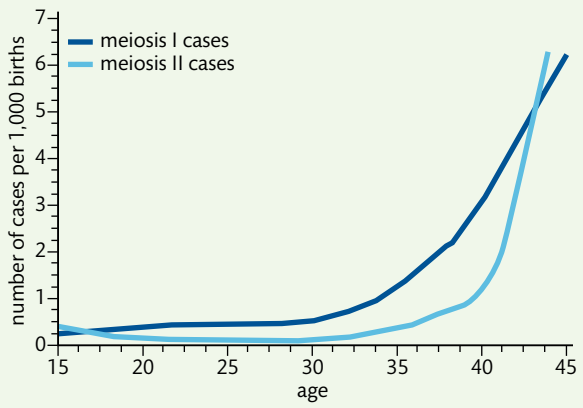
Researchers wanted to find out what influences affected the frequency of Down syndrome. Studies were done by collecting statistics on the many different characteristics of the parents and families of children born with Down syndrome. Such studies are called epidemiological studies, and they look at trends in populations, often examining thousands of cases. Many graphs were made to see if there was a correlation between various factors. The factor that gave the most conclusive results was the age of the mother, as can be seen in the results of one such study shown in Figure 3.20.

The error giving an extra chromosome to the 21st pair can happen during meiosis I or meiosis II, which is why the graph shows both, but the majority of cases are meiosis I. Thanks to such a graph, what advice can doctors give women who wish to avoid this syndrome in their children?



http://biomed.emory.edu/ reproduced with permission

Figure 3.20 Correlation of age of mother and occurrence of Down syndrome in children.

CHALLENGE YOURSELF

Parents who are concerned that they might have a high risk of producing a baby with a chromosomal anomaly (for example a karyotype with 45 or 47 chromosomes instead of 46) may be interested in having a karyogram prepared of the unborn baby's genetic material. This tool has allowed specialists called genetic counsellors to advise parents about their future baby. Genetic counsellors can analyse the karyogram and tell the parents about any chromosomal anomalies such as Down syndrome.

6 What would a genetic counsellor look for in a karyogram to find out if an unborn child has Down syndrome?

Parents who find out that their future child will have a chromosomal disorder that would lead to learning disabilities have a choice to make: some may choose to terminate the pregnancy and try for another child without any anomalies whereas other parents may decide that they will keep the child no matter what.

7 What factors do parents use to make such a difficult decision?

Here is another difficult issue that raises ethical concerns: because a karyogram can be used to determine whether the future baby is a boy or a girl, some parents use this to choose whether they will have the baby. For example, in cultures where having a boy is considered to be more valuable than having a girl (notably in countries where the law prohibits couples from having more than one child), parents might be tempted to terminate pregnancies when the baby is not the sex they want.

8 What would a genetic counsellor look for to determine the sex of the unborn child?

9 In your country, is this an acceptable use of technology?

The two boys in this photo are fraternal twins. The one on the right received an extra 21st chromosome and has Down syndrome.

Obtaining cells for karyotyping

An unborn baby's cells can be extracted in one of two ways: either by a process called amniocentesis or by removing cells from the chorionic villus. Amniocentesis involves using a hypodermic needle to extract some of the amniotic fluid around the developing baby. Inside the liquid, some of the baby's cells can be found and used for the preparation of a karyotype. For the second method, cells are obtained by chorionic villus sampling, which involves obtaining a tissue sample from the placenta's finger-like projections into the uterus wall.

In either case, among the cells collected are foetal cells that are then grown in the laboratory. The preparation of a karyotype is an expensive and invasive procedure. It is usually used for seeing whether an unborn baby has any chromosomal anomalies, e.g. 45 or 47 chromosomes instead of 46. If the parents or doctors are concerned about the chromosomal integrity of an unborn child (for example, if an expectant mother is over the age of 35), a karyotype is recommended.

CHALLENGE YOURSELF

10 Without looking back at the drawings showing them, can you draw the stages of meiosis? Start with a single cell that has two pairs of chromosomes, each having two sister chromatids. In the end, you should have four cells with two single chromosomes in each.

Exercises

6 Look at Figure 3.20. Although the graph clearly shows an increase in the risk of non-disjunction as the mother's age increases, many babies with Down syndrome are born to mothers under the age of 35. Think about it. Can you explain why?

7 Why is meiosis referred to as a reduction division?

8 Explain why meiosis rather than mitosis is necessary for gamete production.

9 State the name of a type of cell in your body that is haploid.

10 Draw and label the stages of meiosis II.

3.4 Inheritance

Understandings:

- Mendel discovered the principles of inheritance with experiments in which large numbers of pea plants were crossed.
- Gametes are haploid so contain only one allele of each gene.
- The two alleles of each gene separate into different haploid daughter nuclei during meiosis.
- Fusion of gametes results in diploid zygotes with two alleles of each gene that may be the same allele or different alleles.
- Dominant alleles mask the effects of recessive alleles but co-dominant alleles have joint effects.
- Many genetic diseases in humans are due to recessive alleles of autosomal genes although some genetic diseases are due to dominant or co-dominant alleles.
- Some genetic diseases are sex linked. The pattern of inheritance is different with sex-linked genes due to their location on sex chromosomes.
- Many genetic diseases have been identified in humans but most are very rare.
- Radiation and mutagenic chemicals increase the mutation rate and can cause genetic diseases and cancer.

Applications and skills:

- Application: Inheritance of ABO blood groups.
- Application: Red–green colour blindness and haemophilia as examples of sex-linked inheritance.
- Application: Inheritance of cystic fibrosis and Huntington's disease.
- Application: Consequences of radiation after nuclear bombing of Hiroshima and accident at Chernobyl
- Skill: Construction of Punnett grids for predicting the outcomes of monohybrid genetic crosses.
- Skill: Comparison of predicted and actual outcomes of genetic crosses using real data.
- Skill: Analysis of pedigree charts to deduce the pattern of inheritance of genetic diseases.

Guidance

- *Alleles carried on X chromosomes should be shown as superscript letters on an upper case X, such as X^h.*
- *The expected notation for ABO blood group alleles is:*

Phenotypes	Genotypes
O	**ii**
A	I^AI^A or I^Ai
B	I^BI^B or I^Bi
AB	I^AI^B

NATURE OF SCIENCE

Making quantitative measurements with replicates to ensure reliability: Mendel's genetic crosses with pea plants generated numerical data.

Gregor Mendel (1822–1884) studied the genetics of garden pea plants.

Mendel's experiments with pea plants

Who was Gregor Mendel?

In 1865, an Austrian monk named Gregor Mendel published the results of his experiments on how garden pea plants passed on their characteristics. At the time,

the term 'gene' did not exist (he used the term 'factors' instead) and the role that DNA played would not be discovered for nearly another century. Some of the questions Mendel asked were:

- How can I be sure that I will get only smooth peas and no wrinkled ones?
- How can I be sure that the resulting plants will be short or tall?
- How can I be sure to obtain only flowers of a certain colour?

NATURE OF SCIENCE

Gregor Mendel used artificial pollination in a series of experiments in which he carefully chose the pollen of various plants to fertilize other individuals of the same species. He used a small brush to place the pollen on the reproductive parts of the flowers, thus replacing the insects that do it naturally. This technique takes away the role of chance because the experimenter knows exactly which plants are fertilized by which pollen.

In one cross, he wanted to see what would happen if he bred tall plants with short plants. The result was that he got all tall plants (see the last row of Table 3.4). But then when he crossed the resulting tall plants with each other, some of the offspring in the new generation were short.

Table 3.4 also shows some of the other characteristics he tried to cross. The × in the first column shows a cross between one variety of pea plant and another. The expected ratio after two generations of crosses is 3:1 (for every 3 of the first type of plant, we would expect 1 of the other type): look how close Mendel got.

Table 3.4 Mendel's results

Characteristics in parents	First generation produced	Second generation produced	Ratio of results seen in second generation
Round × wrinkled seeds	100% round	5474 round 1850 wrinkled	2.96:1
Yellow × green seeds	100% yellow	6022 yellow 2001 green	3.01:1
Green × yellow pods	100% green	428 green 152 yellow	2.82:1
Tall × short plants	100% long	1787 long 277 short	2.84:1

Can you identify the independent variable and dependent variable in each experiment? What about the controlled variables: which things did Mendel make sure were the same from one experiment to the other so that the investigation was a fair test? Does this experiment have the expected characteristics of repeatability and verifiability? Could you do the exact same experiments today, over a century and a half later, and get similar results?

TOK

When Gregor Mendel proposed his ideas about 'factors' (genes) controlling inherited traits, scientists were not eager to adopt his theories. It was not until many decades later, when a new generation of scientists repeated his experiments that the scientific community started to get excited about genetics. What factors influence scientists in their decision to accept or reject new theories?

Also, when examined closely by experts in statistics, some of Mendel's results seem too good to be true. His numbers do not show the expected variations that are typically found by farmers and researchers when breeding plants. What happened? Did he think that the unexpected results were mistakes and so omitted them from his findings? Or did he purposefully change the numbers so they would fit with what he wanted to show? Such a practice is called fudging the data, and it is considered to be unethical. No one knows why Mendel's numbers are so close to perfection, and the mystery may never be solved. How can we be sure that modern scientific studies are free from fudged data?

Key terminology

In order to understand the science of genetics, you first need to know the following terminology.

Genotype – The symbolic representation of the pair of alleles possessed by an organism, typically represented by two letters.

Examples: **Bb**, **GG**, **tt**.

Phenotype – The characteristics or traits of an organism.

Examples: five fingers on each hand, colour blindness, type O blood.

Dominant allele – An allele that has the same effect on the phenotype whether it is paired with the same allele or a different one. Dominant alleles are always expressed in the phenotype.

Example: the genotype **Aa** gives the dominant **A** trait because the **a** allele is masked; the **a** allele is not transcribed or translated during protein synthesis.

Recessive allele – An allele that has an effect on the phenotype only when present in the homozygous state.

Example: **aa** gives rise to the recessive trait because no dominant allele is there to mask it.

Co-dominant alleles – Pairs of alleles that both affect the phenotype when present in a heterozygote.

Example: a parent with curly hair and a parent with straight hair can have children with different degrees of hair curliness, because both alleles influence hair condition when both are present in the genotype.

Locus – The particular position on homologous chromosomes of a gene (as seen in Figure 3.2 and labelled in Figure 3.21). Each gene is found at a specific place on a specific pair of chromosomes.

Homozygous – Having two identical alleles of a gene (see Figure 3.21).

Example: **AA** is a genotype that is homozygous dominant, whereas **aa** is the genotype of someone who is homozygous recessive for that trait.

Heterozygous – Having two different alleles of a gene (see Figure 3.22). This results from the fact that the paternal allele is different from the maternal one.

Example: **Aa** is a heterozygous genotype.

Carrier – An individual who has a recessive allele of a gene that does not have an effect on the phenotype.

Example: **Aa** carries the gene for albinism (like the penguin in the photo on the next page) but has pigmented skin, which means an ancestor must have been albino and some offspring might be albino; if both parents are unaffected by a recessive condition yet both are carriers, some of their progeny could be affected (because they would be **aa**).

Test cross – Testing a suspected heterozygote plant or animal by crossing it with a known homozygous recessive (**aa**). Because a recessive allele can be masked, it is often impossible to tell whether an organism is **AA** or **Aa** unless they produce offspring that have the recessive trait. An example of a test cross is shown later in this section when we explore three generations of pea plants.

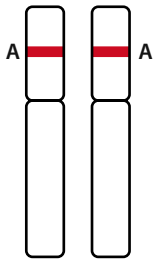


Figure 3.21 This drawing shows you a pair of chromosomes showing a homozygous state, **AA**.

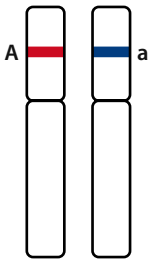


Figure 3.22 This drawing shows you a pair of chromosomes showing a heterozygous state, **Aa**.

Gametes have only one allele of each gene

Constructing a Punnett grid

Figure 3.23 shows a Punnett grid. A Punnett grid can be used to show how the alleles of parents are split between their gametes and how new combinations of alleles can show up in their offspring.

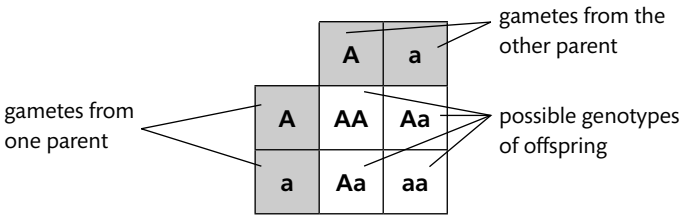


Figure 3.23 A Punnett grid.

The purpose of a Punnett grid is to show all the possible combinations of genetic information for a particular trait in a monohybrid cross. A monohybrid cross is one in which the parents have different alleles and which shows the results for only one trait.

The two alleles of each gene separate

Let's consider a condition called albinism. Most animals are unaffected by albinism and have pigmented skin, hair, eyes, fur, or feathers. But some animals lack pigmentation. An individual with little or no pigmentation is called an albino. For the sake of this illustration, we will assume albinism is controlled by a single gene with two alleles. In reality, the genetics of albinism is more complex, notably because there are multiple types of albinism. However, using our simplification, **A** will represent the allele for pigmentation and **a** will represent the allele for albinism. We can trace the inheritance of albinism with a Punnett grid.

Albino animals lack pigmentation, so this penguin does not have the black markings characteristic of most penguins.

In order to set up a Punnett grid, the following steps must be followed.

1 Choose a letter to show the alleles.

Use the capital and lower case versions of the letter to represent the different alleles. Usually, a capital letter represents the dominant allele and the lower case letter represents the recessive allele. For example:

- **A** = dominant allele, allows pigments to form
- **a** = recessive allele, albinism, allows few or no pigments to form.

Get used to saying 'big A' and 'little a' when reading alleles and genotypes. Also, do not mix letters: for example, you cannot use **P** for pigmented and **a** for albino. Once you have chosen a letter, write down what it means so that it is clear which allele is which.

2 Determine the parents' genotypes.

To be sure that no possibilities are forgotten, write out all three possibilities and decide by a process of elimination which genotype or genotypes fit each parent.

The three possibilities here are:

- homozygous dominant (**AA**) – in this case, the phenotype shows pigmentation
- heterozygous (**Aa**) – in this case, the phenotype shows pigmentation but the heterozygote is a carrier of the albino allele
- homozygous recessive (**aa**) – in this case, the phenotype shows albinism.

The easiest genotype to determine by simply looking at a person or animal is **aa**. The other two are more of a challenge. To determine whether an individual is **AA** or **Aa**, we have to look for evidence that the recessive gene was received from an albino parent or was passed on to the individual's offspring. In effect, the only way to produce an albino is for each parent to donate one **a**.

3 Determine the gametes that the parents could produce.

An individual with a genotype **AA** can only make gametes with the allele **A** in them. Heterozygous carriers can make **A**-containing gametes or **a**-containing gametes. Obviously, individuals whose genotype is **aa** can only make gametes that contain the **a** allele. So you can record and label with **A** or **a** all the possible gametes.

4 Draw a Punnett grid.

Once all the previous steps have been completed, drawing the actual grid is simple. The parents' gametes are placed on the top and side of the grid. As an example, consider a cross involving a female carrier **Aa** crossed with a male albino **aa**.

You might guess that, because there are three **a** alleles and only one **A**, there should be a three out of four chance of seeing offspring with the recessive trait. But this is not the case. Figure 3.24 is a grid with the parents' gametes.

	a	a
A		
a		

Figure 3.24 A Punnett grid showing the parent's gametes.

Now you can fill in the empty squares with each parent's possible alleles by copying the letters from the top down and from left to right. When letters of different sizes end up in the same box, the big one goes first.

	a	a
A	Aa	Aa
a	aa	aa

Figure 3.25 A Punnett grid with all the possible genotypes filled in.

Be careful when choosing letters. Nearly half the letters of the alphabet should in fact be avoided because they are too similar in their capital and lower case forms. Don't use Cc, Ff, Kk, Oo, Pp, Ss, Uu, Vv, Ww, Xx, Yy, Zz.

The five steps of the Punnett grid method.

- **Step 1 – Choose a letter.**

  T = allele for a tall plant.

  t = allele for a short plant.
- **Step 2 – Parents' genotypes.**

  TT for the purebred tall parent.

  tt for the purebred short parent.
- **Step 3 – Determine gametes.**

  The purebred tall parent can only give T.

  The purebred short parent can only give t.
- **Step 4 – Draw a Punnett grid.**

	t	t
T	Tt	Tt
T	Tt	Tt

Figure 3.26 A Punnett grid for **TT** and **tt**.

- **Step 5 – Interpret grid.**

  100% Tt and will be tall, so 0% will be short.

When answering questions about genetic outcomes for offspring, it is sometimes tempting to go straight to the Punnett grid and forget about steps 1–3. The problem is that if you do not think carefully about the information going into the Punnett grid, you could put in the wrong information.

5 Work out the chances of each genotype and phenotype occurring.

In a grid with four squares, each square can represent one of two possible statistics:

- the chance that these parents will have offspring with that genotype, here each square represents a 25% chance
- the probable proportion of offspring that will have the resulting genotypes, this only works for large numbers of offspring.

Fusion of gametes

The results from the above example show the following: there is a 50% chance of producing offspring with genotype **Aa** and a 50% chance of producing offspring with genotype **aa**. Because humans tend to produce a small number of offspring, this is the interpretation that should be used. If the example was about plants that produce hundreds of seeds, the results could be interpreted in the following way: 50% of the offspring should be **Aa** and the 50% should be **aa**.

No matter what the outcome, each offspring is the result of two alleles coming together when the gametes fuse. In this process, the two haploid sex cells join to make a single diploid cell called a zygote. This is the first cell of the new offspring.

Finally, the phenotypes can be deduced by looking at the genotypes. For example, **Aa** offspring will have a phenotype showing pigmentation so they will not be affected by albinism, whereas all the **aa** offspring will be albinos.

Dominant alleles and co-dominant alleles

Using the five steps of the Punnett grid method, we are going to examine the theoretical chances of genetic traits being passed on from one generation to the next.

Short or tall pea plants?

Let's first consider a cross that Gregor Mendel did with his garden pea plants. He took purebred tall plants and crossed them with purebred short plants. Purebred means that the tall plants' parents were known to be all tall, and the short plants' parents were known to be all short. In other words, he knew that none of the plants was heterozygous. He wanted to find out whether he would get all tall plants, some tall and some short, or all short.

The answer took months for Mendel to confirm, but a Punnett grid can now be used to get the answer in seconds: the result was 100% tall plants. Why? Because in garden pea plants, the allele for tall is dominant over the allele for short plants, thus masking the short trait in heterozygotes.

The name given to the generation produced by a cross such as this is the first filial generation, usually referred to as the F_1 generation. What would happen if tall plants from the F_1 generation were crossed to make a second filial generation (F_2)? A Punnett grid can give us the results.

	T	**t**
T	**TT**	**Tt**
t	**Tt**	**tt**

Figure 3.27 A second filial generation.

This grid can be interpreted in two ways:

- there is a 75% chance of producing tall offspring and a 25% chance of producing short offspring
- 75% of the offspring will be tall and 25% of the offspring will be short.

Although 75% of the plants are tall, they have differing genotypes. Some tall plants are homozygous dominant and others are heterozygous.

Also, in a real experiment, it is unlikely that exactly 25% of the offspring would be short plants. The reason is essentially due to chance. For example, if 90 F_2 peas were produced and all of them were planted and grew into new plants, there is no mathematical way that exactly 25% of them would be short. At the very best, 23 out the 90 plants would be short , which is 25.56%; that is as close as it is possible to get to 25% in this case.

Even if a convenient number of plants was produced, such as 100 plants, farmers and breeders would not be surprised if they got 22, 26 or even 31 short plants instead of the theoretical 25. If the results of hundreds of similar crosses were calculated, the number would probably be very close to 25%. The same phenomenon can be seen in the sex of human children. Although the theoretical percentage is calculated to be 50% girls and 50% boys, in reality few families have exactly half and half. The actual result is due to chance.

Test cross

A plant breeder might need to know whether a specific tall plant from the F_2 generation is a purebred for tallness (homozygous dominant, **TT**) or whether it will not breed true for tallness (heterozygous **Tt**). To find out, she would cross the tall plant (whose genotype is not known) with a plant whose genotype is definitely known: a short plant that must be homozygous recessive, **tt**. By looking at the resulting plants, the test cross can reveal the genotypes of the tall plant as either **TT** or **Tt**.

If she gets a mix of tall and short plants as a result of the cross, she can conclude that the tall plant is heterozygous. The Punnett grid in Figure 3.28 explains her reasoning.

	t	t
T	Tt	Tt
t	tt	tt

Figure 3.28 Test cross between a heterozygous tall plant and a homozygous recessive short plant.

If, on the other hand, all the offspring are tall, without exceptions, she can conclude that the tall plant is **TT**. The Punnett grid would be identical to the one in Figure 3.26. There is another possible interpretation to these results, however. The tall plant could, in fact, be **Tt** but by chance it only passed on **T** and never passed on **t**. Although this is possible, it is unlikely in cases where many offspring are produced.

Multiple alleles

So far, only two possibilities have been considered for a gene: dominant, **A**, or recessive, **a**. With two alleles, three different genotypes are possible, which can produce two different phenotypes. However, genetics is not always this simple;

sometimes there are three or more alleles for the same gene. This is the case for the alleles that determine the ABO blood type in humans.

Blood type: an example of multiple alleles

The ABO blood type system in humans has four possible phenotypes: A, B, AB and O. To create these four blood types there are three alleles of the gene. These three alleles can produce six different genotypes.

The gene for the ABO blood type is represented by the letter **I**. To represent more than just two alleles (**I** and **i**) superscripts are introduced. As a result, the three alleles for blood type are written as follows: $\mathbf{I^A}$, $\mathbf{I^B}$ and **i**. The two capital letters with superscripts represent alleles that are co-dominant:

- $\mathbf{I^A}$ = the allele for producing proteins called type A antigens, giving type A blood
- $\mathbf{I^B}$ = the allele for producing proteins called type B antigens, giving type B blood
- **i** = the recessive allele that produces neither A nor B antigens, giving type O blood.

Crossing these together in all possible combinations creates six genotypes that give rise to the four phenotypes listed earlier:

- $\mathbf{I^AI^A}$ or $\mathbf{I^Ai}$ gives a phenotype of type A blood
- $\mathbf{I^BI^B}$ or $\mathbf{I^Bi}$ gives type B blood
- $\mathbf{I^AI^B}$ gives type AB blood (because of co-dominance, both types of antigens are produced)
- **ii** gives type O blood.

Notice how the genotype $\mathbf{I^AI^B}$ clearly shows co-dominance. Neither allele is masked: both are expressed in the phenotype of type AB blood.

Worked example

Is it possible for a couple to have four children, each child showing a different blood type?

Solution

There is only one way for this to happen: one parent must have type A blood but be a carrier of the allele for type O blood, and the other parent must have type B blood and also be a carrier of the allele for type O blood (if necessary, remind yourself of the blood group alleles, as shown above).

The cross would be $\mathbf{I^Ai \times I^Bi}$ and the grid is shown in Figure 3.29. See if you can determine the phenotype of each child before reading on.

So, would it be possible for this couple to have four children and all of them have a different blood group? In theory, yes.

Would it be possible for the same couple to have four children and all of them have type AB blood? In theory, yes, but it would not be likely. This question is similar to asking 'Could a couple have 10 children, all of them girls?' It is possible but statistically unlikely.

	$\mathbf{I^A}$	**i**
$\mathbf{I^B}$	$\mathbf{I^AI^B}$	$\mathbf{I^Bi}$
i	$\mathbf{I^Ai}$	**ii**

Figure 3.29 A Punnet grid for blood type alleles.

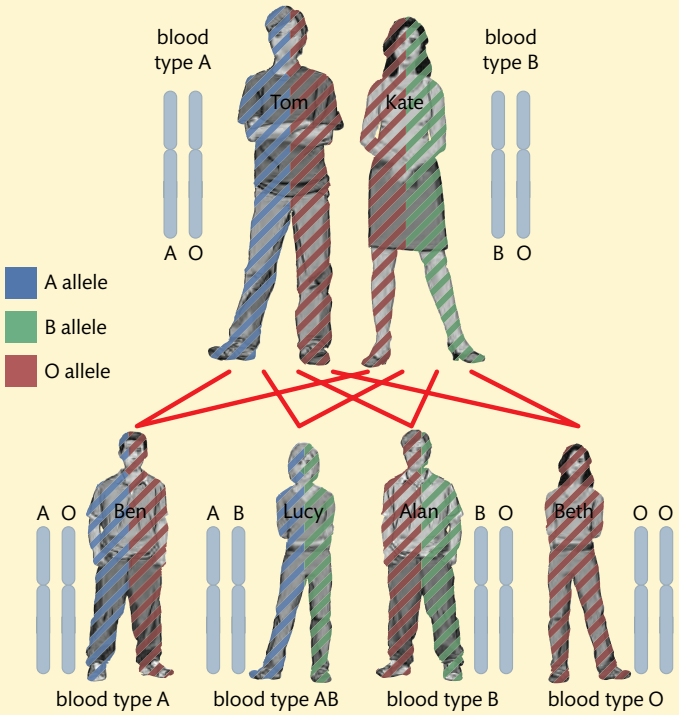


Figure 3.30 How the ABO blood groups can be inherited.

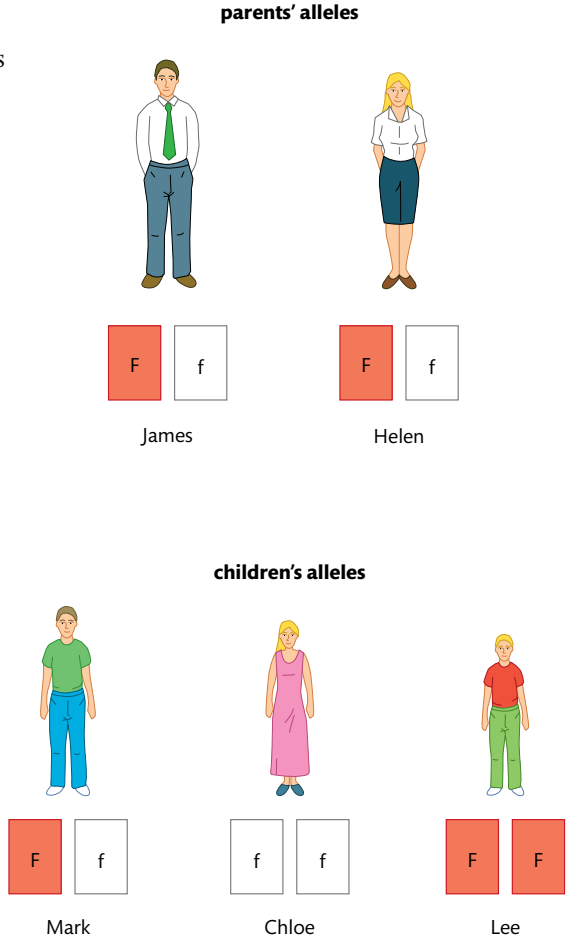
Figure 3.31 Cystic fibrosis inheritance.

Autosomal genetic diseases in humans

How is it possible for two healthy parents to have a child who suffers from a genetic disease? You should understand enough about how genetics works to be able to answer this: it's because the disease is recessive and both healthy parents must be carriers of the allele that causes the disease. For example, in the case of cystic fibrosis, let's call **F** the allele that leads to healthy production of mucus and **f** the allele for cystic fibrosis. In Figure 3.31, showing a family that has cystic fibrosis, the parents James and Helen are carriers (**Ff**). The only way to have the disease is to have the genotype **ff**, so James and Helen do not suffer from cystic fibrosis but they can pass it on to their children. If you set up a Punnett grid for these parents, you will see that there is a 1 in 4 chance (25%) that they will have a child with cystic fibrosis, and there are three possibilities for the genotypes in their children: Mark is **Ff**, Chloe is **ff**, and Lee is **FF**.

Such diseases are called autosomal recessive diseases because they are caused by recessive alleles, and the locus of their gene is found on one of the first 22 pairs of chromosomes but not on the sex chromosomes X or Y. The following are examples of autosomal recessive diseases:

- albinism
- cystic fibrosis
- phenylketonuria (PKU)
- sickle cell disease and sickle cell trait
- Tay Sachs disease
- thalassemia.



Genetic diseases are rare

You have probably heard of some of the conditions listed above, but not all, and it is unlikely that you will encounter any more than a handful of people with these diseases in your lifetime, because they are so rare in the general population. Even the most frequently occurring autosomal recessive diseases only affect about 1 in 2000 people in a given population, others typically as few as 1 in 10 000 or 20 000 people.

NATURE OF SCIENCE

Students sometimes get the impression that genetics is only about diseases. This is not true. It's just that more is known about disease-causing genes than about things such as eye colour genes, because researchers spend their time and funds studying things that can help society. Studying diseases and discovering their genetic causes is more useful to medicine than studying eye colour. Governments and university laboratories investing money in research want their work and their discoveries to lead to healthier lives for people. Getting a return on their investment also motivates them. Fundamental research ('I would like to study this just to find out how it works') does not attract funding as much as applied research ('I would like to find out how this disease is caused so that we can find better medical treatments for it').

Diseases caused by sex-linked genes or co-dominant alleles

Genes carried on the sex chromosomes

Because the Y chromosome is significantly smaller than the X chromosome, it has fewer loci and therefore fewer genes than the X chromosome. This means that sometimes alleles present on the X chromosome have nothing to pair up with. For example, a gene whose locus is at an extremity of the X chromosome would have no counterpart on the Y chromosome because the Y chromosome does not extend that far from its centromere.

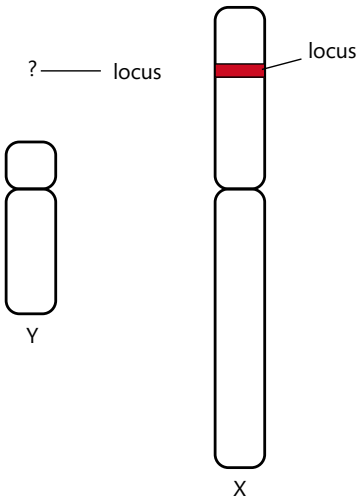


Figure 3.32 Since the Y chromosome is smaller than the X chromosome, there are fewer loci. As a result, the locus marked with a red bar on the X chromosome does not exist on the Y chromosome

Sex linkage

Any genetic trait whose gene has its locus on the X or the Y chromosome is said to be sex linked. Often genetic traits that show sex linkage affect one sex more than the other. Two examples of genetic traits that have this particularity are colour blindness and haemophilia.

- Colour blindness is the inability to distinguish between certain colours, often green and red. To people who are colour blind, these two colours look the same; they would not see a difference between a green apple and a red apple, for example.
- Haemophilia is a disorder in which blood does not clot properly. For most people, a small cut or scrape on their skin stops bleeding after a few minutes and eventually a scab forms. This process is called clotting. People with haemophilia have trouble with blood clotting and are at risk of bleeding to death from what most people would consider to be a minor injury such as a bruise, which is a rupture of many tiny blood vessels. Such bleeding can also occur in internal organs. Medical treatments such as special injections help give people affected by haemophilia a better quality of life.

Alleles and genotypes of sex-linked traits

Because the alleles for both colour blindness and haemophilia are found only on the X chromosome, the letter X is used when representing them:

- $\mathbf{X^b}$ = allele for colour blindness
- $\mathbf{X^B}$ = allele for the ability to distinguish colours
- $\mathbf{X^h}$ = allele for haemophilia
- $\mathbf{X^H}$ = allele for the ability to clot blood
- **Y** = no allele present on the Y chromosome.

As there is no allele on the Y chromosome, Y is written alone without any superscript. Here are all the possible genotypes for colour blindness:

- $\mathbf{X^BX^B}$ gives the phenotype of a non-affected female
- $\mathbf{X^BX^b}$ gives the phenotype of a non-affected female who is a carrier
- $\mathbf{X^bX^b}$ gives the phenotype of an affected female
- $\mathbf{X^BY}$ gives the phenotype of a non-affected male
- $\mathbf{X^bY}$ gives the phenotype of an affected male.

In the above list, ^{B} and ^{b} could be replaced by ^{H} and ^{h} to show the genotypes for haemophilia. Notice how only one sex can be a carrier.

The letters X and Y refer to chromosomes and not to alleles, so terms such as dominant and recessive do not apply. X and Y should be considered as entire chromosomes rather than alleles of a gene. In sex-linked alleles, the letter that indicates the allele is the superscript after the X or Y. An absence of a superscript means that no allele for that trait exists on that chromosome.

The pattern of inheritance with sex-linked genes

Carriers of sex-linked traits

Sex-linked recessive alleles such as $\mathbf{X^b}$ are rare in most populations of humans worldwide. For this reason, it is unlikely to get one and much less likely to get two such alleles. This is why so few women are colour blind: their second copy of the gene is likely to be the dominant allele for full colour vision and will mask the recessive allele. The same is true for haemophilia.

As you have seen, there are three possible genotypes for females but only two possible genotypes for males. Only women can be heterozygous, $\mathbf{X^BX^b}$, and, as a result, they are the only ones who can be carriers.

Because men do not have a second X chromosome, there are only two possible genotypes, $\mathbf{X^BY}$ or $\mathbf{X^bY}$, for them in relation to colour blindness. With just the one recessive allele **b**, a man will be colour blind. This is contrary to what you have seen up to now concerning recessive alleles: usually people need two to have the trait, and, with one, they are carriers. In this case, the single recessive allele in males determines the phenotype. Men cannot be carriers for X-linked alleles.

As well as colour blindness and haemophilia, more examples of sex-linked traits in humans and other animals include:

- Duchene muscular dystrophy
- white eye colour in fruit flies
- calico–tortoiseshell fur colour in cats.

Because the scientist John Dalton had red–green colour blindness, the condition is sometimes referred to as Daltonism and people who have it are said to be Daltonian. Dalton asked for his eyes to be dissected after his death (he died in 1844) to verify his hypothesis that the liquid inside them was blue. It was not. However, his eyes were kept for study, and, a century and a half later, scientists used the tissue samples to identify the gene for colour blindness.

Worked example

The term 'pedigree' refers to the record of an organism's ancestry. Pedigree charts are diagrams that are constructed to show biological relationships. In genetics, they are used to show how a trait can pass from one generation to the next. Used in this way for humans, a pedigree chart is similar to a family tree, complete with parents, grandparents, aunts, uncles, and cousins.

To build such a chart, symbols are used to represent people. Preparing a pedigree chart helps prepare Punnett grids for predicting the probable outcome for the next generation.

Example 1: Huntington's disease

Huntington's disease (Huntington's chorea) is caused by a dominant allele that we will refer to by the letter **H**. This genetic condition causes severely debilitating nerve damage but the symptoms do not show until the person is about 40 years old. As a result, someone who has the gene for Huntington's disease may not know it for certain until they have started a career and possibly started a family.

The symptoms of Huntington's disease include difficulty walking, speaking, and holding objects. Within a few years of starting the symptoms, the person loses complete control of his or her muscles and dies an early death. Because it is dominant, all it takes is one **H** allele in a person's genetic makeup to cause the condition.

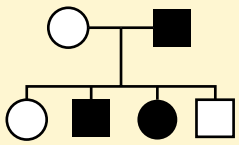


Figure 3.33 This is a pedigree chart showing members of a family affected by Huntington's disease.

1 Give a full description of the six individuals in Figure 3.33, saying who is affected and who is not.

2 State the genotype for each individual.

Solution

1 The symbols indicate that the unaffected members of the family are the mother, the first child (a girl) and the fourth child (a boy). Those who are affected are the father, the second child (a boy) and the third child (a girl).

2 To work out if the father is **HH** or **Hh**, consider the fact that some of his children do not have the trait. This proves that he must have given one **h** to each of them. Hence, he can only be **Hh** and not **HH**. The mother is not affected so she must be **hh**. This is also true for the first daughter and the last son. Since the mother always gives an **h**, the two middle children must have at least one **h**, but, because they are affected, they are **Hh.**

Example 2: co-dominance in flower colour

Co-dominance in certain flowers can create more than two colours, so a pedigree chart can help keep track of how the offspring got their phenotypes. For example, in purebred snapdragon flowers, sometimes white × red = pink.

The system of letters for showing colour in snapdragon flowers uses a prefix **C**, which refers to the gene that codes for flower colour, plus a superscript, which refers to the specific colour, **R** (red) or **W** (white).

These are the symbols used in pedigree charts.

- **○ empty circle = female**
- **□ empty square = male**
- **● filled-in circle = a female who possesses the trait being studied**
- **■ filled-in square = a male who possesses the trait being studied**
- **| vertical line = the relationship parents and offspring**
- **– horizontal line between a man and a woman = they are the parents who had the offspring**

So the alleles for co-dominant flower colour are:

- C^R for red flowers
- C^W for white flowers.

The genotypes and their phenotypes are:

- C^RC^R makes red flowers
- C^WC^W makes white flowers
- C^RC^W makes pink flowers.

For co-dominant traits, grey is used in pedigree charts rather than black or white.

1 Using the pedigree chart below, state the genotypes for all the plants A to K.

2 What evidence is there that genetic characteristics can sometimes skip a generation?

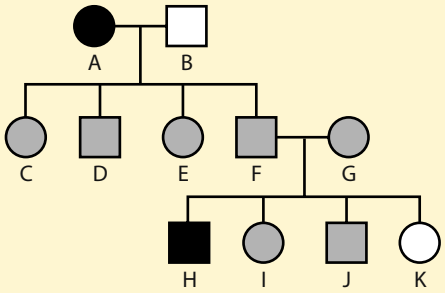


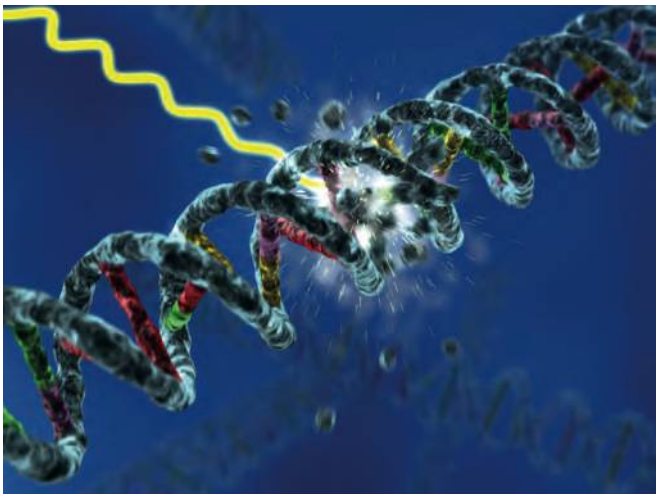
Figure 3.34 This pedigree chart shows how pink flowers can arise in purebred snapdragon plants. Black shapes represent snapdragon plants with red flowers, white shapes represent white-flowered plants and grey shapes represent plants with pink flowers.

Solutions

1 A and H produce red flowers and must be homozygous for red, C^RC^R, because any other combination would give pink or white. B and K produce white flowers and must be homozygous for white, C^WC^W, because any other combination would give pink or red. C to G as well as I and J are pink and must be heterozygous, C^RC^W, because they have one of each allele from each parent plant.

2 It would be impossible for either the colour red or the colour white to be in the middle generation in this diagram. These colours skip a generation and show up again in the last row.

Some possible causes of mutations, genetic diseases and cancer



An artist's conception of how DNA can be damaged by radiation.

Although it is beyond the scope of this chapter, it is interesting to note that the science of epigenetics challenges the idea that genetics is unchangeable during the lifetime of an individual. In some cases, environmental factors during an organism's lifetime can have an influence in turning on or turning off certain genes.

In principle, DNA is not supposed to be modified during the lifetime of an individual. Normally, the code should be preserved. However, there are exceptions, and exposure to radiation or to carcinogens (cancer-causing chemicals) can sometimes modify the code and cause serious health threats.

NATURE OF SCIENCE

When and how did we find out that X-rays were not a safe and healthy way of performing prenatal (during pregnancy) examinations?

Dr Alice Stewart was particularly talented with numbers. She knew the power of statistical analysis in determining correlation and was horrified by what she found when studying the records of infants dying of cancer. Her statistical analysis in the early 1950s demonstrated that children whose mothers had prenatal X-rays were twice as likely to die of cancer than children whose mothers did not have prenatal X-rays. Although the studies were scientifically sound and the statistics were reliable, doctors did not accept them at first and continued to use X-rays on pregnant women for more than two decades. Stewart was criticized for her work and had trouble getting funding for subsequent projects. Only in the 1970s did other scientists repeat studies similar to hers, with the same results, and finally X-rays were replaced with non-radioactive techniques such as ultrasound sonograms.

What does this case study reveal about the nature of science? And about the importance of repeatability and verifiability? What prevented doctors from taking action immediately and stopping the use of X-rays on pregnant women in the 1950s? Often people use expressions like 'the numbers don't lie' when talking about statistics. Is this always the case? Looking back, it might be tempting to say that doctors using X-rays on pregnant women after Stewart's report were acting unethically: what do you think?

Other causes of cancer and disease

Diseases such as cancer can sometimes be caused by mutagenic chemicals. Chemistry teachers will tell you that the list of products they are allowed to use with their students today is different compared with when they were students: products such as benzene, which were commonly used in laboratories in the past, are now restricted or forbidden because of their cancer-causing or mutagenic properties. Such chemicals, in high concentrations and with long exposure times, can cause mutations and cancer just as radioactivity can: in a silent and invisible way.

Should we be worried? Toxic things in our environment are regulated by government standards. Normally, as long as the concentrations and exposure times are respected, the danger to your health is very limited. The problem is, sometimes people do not know or do not follow the recommendations for the products they use. Also, companies often test a product alone but not necessarily in conjunction with other products. A pesticide that a woman puts on her vegetable garden may not cause cancer in the doses she inhales or gets on her skin, but if she smokes and she works at the radiology department in the local hospital, and she uses a cell phone many hours a day, and she goes to the tanning salon regularly, and she lives in a city with severe air pollution … Could all those repeated non-lethal daily doses of possible cancer-causing things add up? Or compound each other? These are complex questions that require conclusive evidence in order to be able to say one way or the other.

DNA and radiation

As the early experimenters with radium found out, radioactivity can cause cancer. Not knowing of the dangers when she was studying radium, the pioneer Marie Curie, the first person to win two Nobel prizes, carried samples of radioactive materials

around with her, and kept them on laboratory tables without any precautions. Not surprisingly, she died of leukaemia, and her laboratories, which you can visit in Paris, still show radioactive contamination today.

Marie Curie, who discovered the radioactive elements polonium and radium, did not benefit from the safety standards we have today, and died at the age of 66 from her exposure to radioactivity.

The world saw the terrifying effects of radiation poisoning on people when the city of Hiroshima was the target of the first atomic bomb used in warfare in August 1945. It is estimated that 100 000 people died at its impact or shortly after, but it is difficult to estimate how many died later from the effects of radiation in the city.

When radiation hits a DNA molecule, it can sometimes knock one or more base pairs out of place, modifying the genetic code. This causes a mutation that, as we have seen, can sometimes be benign (not harmful), but at other times it can be harmful to an organism. When the DNA mutation leads to cancer, as happened to Marie Curie, the organism's health is in jeopardy. However, Marie Curie's husband, Pierre Curie, did not die of cancer, but of something equally dangerous: he slipped in the street and was run over by a horse-drawn carriage in Paris in 1906.

Besides nuclear bombs, another source of radiation is nuclear power plants. As long as they are safe and secure, there should not be any risk of radiation leaking out into the environment. There have been some cases in recent history, however, that have revealed the potential dangers of nuclear power plants: Chernobyl in 1986 and Fukushima in 2011 are two such examples. In both situations, radioactive material was leaked out into the environment and the zones around the out-of-commission power plants were evacuated of all human populations within a radius of tens of kilometres.

Ecology experts studying the area around Chernobyl.

Ecologists are studying the area around Chernobyl to see how nature has responded to the presence of radiation. In some instances, the scientists have been pleasantly surprised to find that nature seems to be doing fine despite the dangerously high

radiation levels. In other instances, they have confirmed the presence of mutations in the plants and animals that have colonized the abandoned zone. Cancer studies in the peripheral zones where people are allowed to live, beyond 30 km from the shut-down Chernobyl reactor, suggest that there has been an increase in cancer frequencies. The nuclear power industry has made an effort to isolate the abandoned nuclear power plant at Chernobyl by encasing it in a dome of cement. The hope is that the cement will be thick enough to stop the radiation from continuing to escape into the environment.

Exercises

11 Explain why more men are affected by colour blindness than women.

12 Using the C^R and C^W alleles for co-dominance in snapdragon flower colour, show how two plants could have some white-flowered offspring, some pink-flowered offspring and some red-flowered offspring within one generation.

13 Draw a pedigree chart of the two generations described in question 12.

14 Look at the grid below showing the chances that a couple's children might have haemophilia.

(a) State the genotype of the mother and father.
(b) State the possible genotypes of the girls and boys.
(c) State the phenotypes of the girls and boys.
(d) Who are the carriers in this family?
(e) What are the chances that the parents' next child will be a haemophiliac?

	X^H	Y
X^H	X^HX^H	X^HY
X^h	X^HX^h	X^hY

NATURE OF SCIENCE

Assessing risks associated with scientific research: scientists attempt to assess the risks associated with genetically modified crops or livestock.

3.5 Genetic modification and biotechnology

Understandings:

- Gel electrophoresis is used to separate proteins or fragments of DNA according to size.
- PCR can be used to amplify small amounts of DNA.
- DNA profiling involves comparison of DNA.
- Genetic modification is carried out by gene transfer between species.
- Clones are groups of genetically identical organisms derived from a single original parent cell.
- Many plant species and some animal species have natural methods of cloning.
- Animals can be cloned at the embryo stage by breaking up the embryo into more than one group of cells.
- Methods have been developed for cloning adult animals using differentiated cells.

Applications and skills:

- Application: Use of DNA profiling in paternity and forensic investigations.
- Application: Gene transfer in bacteria using plasmids makes use of restriction endonucleases and DNA ligase.
- Application: Assessment of the potential risks and benefits associated with genetic modification of crops.
- Application: Production of cloned embryos produced by somatic-cell nuclear transfer.
- Skill: Design of an experiment to assess one factor affecting the rooting of stem cuttings.
- Skill: Analysis of examples of DNA profiles.
- Skill: Analysis of data on risks to monarch butterflies of Bt crops.

Guidance

- *Students should be able to deduce whether or not a man could be the father of a child from the pattern of bands on a DNA profile.*
- *Dolly can be used as an example of somatic-cell transfer.*
- *A plant species should be chosen for rooting experiments that forms roots readily in water or a solid medium.*

Exploring DNA

DNA is at the very core of what gives animals and plants their uniqueness. We are now going to look at the astounding genetic techniques, developed during the past few decades, that enable scientists to explore and manipulate DNA. These include:

- copying DNA in a laboratory – the polymerase chain reaction (PCR)
- using DNA to reveal its owner's identity – DNA profiling
- mapping DNA by finding where every A, T, C, and G is – gene sequencing, including the Human Genome Project
- cutting and pasting genes to make new organisms – gene transfer
- cloning cells and animals.

These techniques offer new hope for obtaining treatments and vaccines for diseases; for creating new plants for farmers; for freeing wrongly convicted people from prison by proving their innocence with DNA tests.

Techniques such as gene transfer and cloning have sparked heated debates. Is it morally and ethically acceptable to manipulate nature in this way? Are the big biotech companies investing huge sums of money into this research to help their fellow citizens, or are they just in it for the economic profit? Concerning cloning and stem cell research, is it morally and ethically acceptable to create human embryos solely for scientific research?

Part of being a responsible citizen is making informed decisions relating to these difficult questions. It is not just technical complexity that makes these questions difficult, it is also because we have never had to face them before.

Gel electrophoresis

This laboratory technique is used to separate fragments of DNA in an effort to identify its origin. Enzymes are used to chop up the long filaments of DNA into varying sizes of fragments. The DNA fragments are placed into small wells (holes) in the gel, which are aligned along one end. The gel is exposed to an electric current, positive on one side and negative on the other.

The effect is that the biggest, heaviest, and least charged particles do not move easily through the gel, so they get stuck very close to the wells they were in at the beginning. The smallest, least massive, and most charged particles pass through the gel to the other side with little difficulty. Intermediate particles are distributed in between. In the end, the fragments leave a banded pattern of DNA like the one shown in the photo.

As seen in Figure 3.35, gel electrophoresis can stop there or a hybridization probe can be added. A probe, in this case for sickle cell disease, is a known sequence of a complementary DNA sequence that binds with a DNA strand in the gel, revealing the presence of the gene we are interested in.

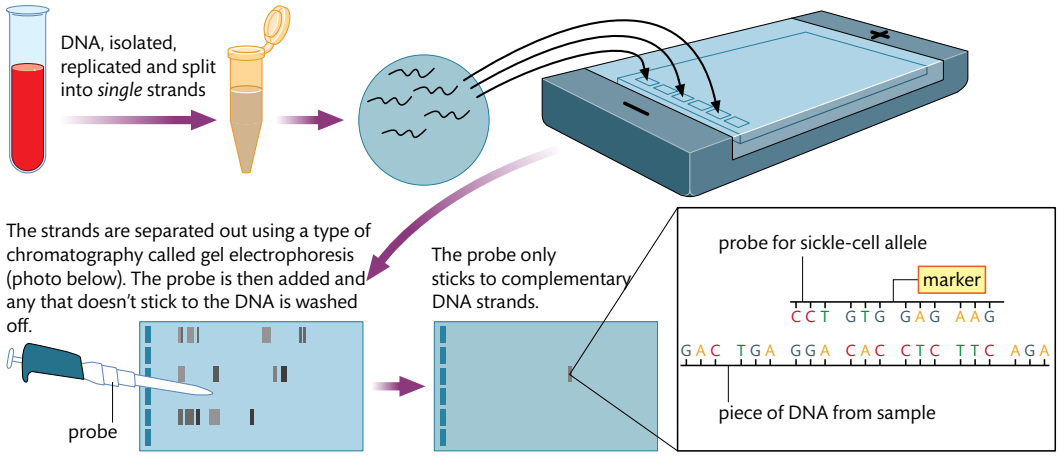


Figure 3.35 Gel electrophoresis is used to separate DNA fragments so that they can be analysed.

This autoradiogram (or autoradiograph) shows banded lines that were formed from nine different DNA samples during gel electrophoresis. The black traces are left by the radioactivity of the materials used in marking the DNA samples.

CHALLENGE YOURSELF

11 Based on the evidence shown in the autoradiogram to the right, deduce which child (C_1, C_2, or C_3) is most likely to be the child of the father whose track is being pointed to (F). The mother is in the first track on the far left. Justify your answer.

PCR: how to make lots of copies of DNA

Polymerase chain reaction (PCR)

PCR is a laboratory technique using a machine called a thermocycler that takes a very small quantity of DNA and copies all the nucleic acids in it to make millions of copies of the DNA (see Figure 3.36). PCR is used to solve the problem of how to get enough DNA to be able to analyse it.

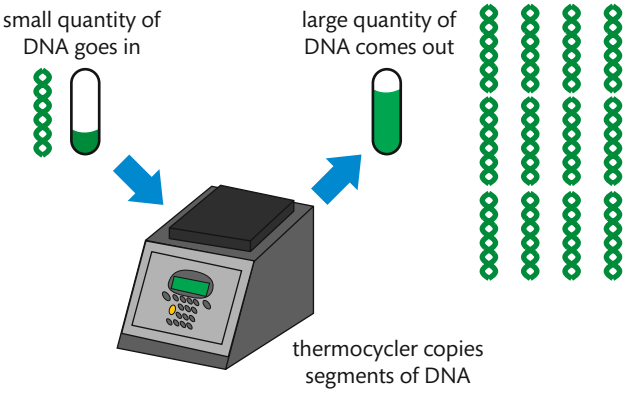


Figure 3.36 Analysis is impossible with the DNA from just one or a few cells. PCR is a way of ensuring that enough DNA for analysis can be generated.

When collecting DNA from the scene of a crime or from a cheek smear, often only a very limited number of cells are available. By using PCR, forensics experts or research technicians can obtain millions of copies of the DNA in just a few hours. Such quantities are large enough to analyse, notably using gel electrophoresis.

DNA profiling

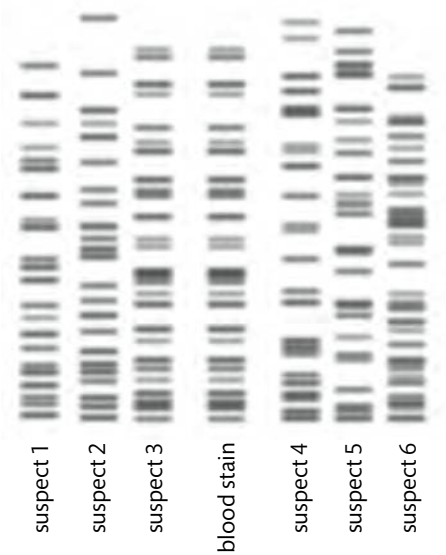
The process of matching an unknown sample of DNA with a known sample to see if they correspond is called DNA profiling. This is also sometimes referred to as DNA fingerprinting because there are some similarities with identifying fingerprints, but the techniques are very different.

If, after separation by gel electrophoresis, the pattern of bands formed by two samples of DNA fragments are identical, it means that both most have come from the same individual. If the patterns are similar, it means that the two individuals are probably related.

Applications of DNA profiling

DNA profiling can be used in paternity suits when the identity of someone's biological father needs to be known for legal reasons.

At a crime scene, forensics specialists can collect samples such as blood or semen, which contain DNA. Gel electrophoresis is used to compare the collected DNA with that of suspects. If they match, the suspect has a lot of explaining to do. If there is no match, the suspect is probably not the person the police are looking for. Criminal cases are sometimes reopened many years after a judgement was originally made, in order to consider new DNA profiling results. In the USA, this has led to the liberation of many individuals who had been sent to jail for crimes they did not commit.



These seven tracks were produced by gel electrophoresis to allow investigators to analyse and match DNA samples.

CHALLENGE YOURSELF

12 Using the adjacent DNA profiles from six suspects, can you identify which one matches the DNA profile of the blood stain found at the crime scene?

DNA profiling is used in other circumstances too, for example in studies of ecosystems, when scientists use DNA samples taken from birds, whales, and other organisms to clarify relationships. This has helped establish a better understanding of social relationships, migrating patterns, and nesting habits, for example. In addition,

TOK

- How do you think a child would feel if she were to find out from DNA profiling that her father was not her biological father?
- How would a man feel if he found out he was not his child's father?
- What effect would such a result have on the relationships between siblings or between spouses?
- What kind of emotions might someone feel after spending 18 years in prison, and then being freed thanks to a DNA test?

the study of DNA in the biosphere has given new credibility to the ideas of evolution: DNA evidence can often reinforce previous evidence of common ancestry based on anatomical similarities between species.

How DNA profiles are analysed

In the photo on page 158, showing gel electrophoresis of nine samples of DNA, the line marked C_2 (child number 2) and the one being pointed to, F (father), show similarities in their banding patterns. However, the children marked C_1, C_3, and C_4 do not show many similarities.

From this DNA evidence, it should be clear that person F is much more likely to be the father of child number 2 than of any of the other children. Similar techniques are used to analyse the similarities and differences between DNA collected at a crime scene and DNA samples taken from suspects.

The techniques have been perfected to a point where it is possible to determine the identity of someone by examining cells found in the traces of saliva left on the back of a postage stamp on a letter.

TOK

How do we decide when evidence is reliable or not? Often when DNA evidence is used in a courtroom trial, it has a certain credibility as scientific fact, and yet we know from our own experience in lab work that there is a degree of error in any procedure. Whether it be in the laboratory or in a courtroom, it is difficult to imagine evidence that can be considered 100% certain. When a scientist comes up with new evidence, old theories can be challenged or even overturned. But how do we decide which evidence is to be accepted and which evidence is to be discarded?

Genetic modification: gene transfer between species

Gene transfer

The technique of taking a gene out of one organism (the donor organism, e.g. a fish) and placing it in another organism (the host organism, e.g. a tomato) is a genetic engineering procedure called gene transfer. Just such a transfer was done to make tomatoes more resistant to cold and frost.

It is possible to put one species' genes into another's genetic makeup because DNA is universal: as you will recall (Section 2.6), all known living organisms use the bases A, T, C, and G to code for proteins. The codons they form always code for the same amino acids, so transferred DNA codes for the same polypeptide chain in the host organism as it did in the donor organism. In the example above, proteins used by fish to resist the icy temperatures of arctic waters are now produced by the modified tomatoes to make them more resistant to cold.

Another example of gene transfer is found in Bt corn, which has been genetically engineered to produce toxins that kill the bugs that attack it. The gene, as well as the name, comes from a soil bacterium, *Bacillus thuringiensis*, which has the ability to produce a protein that is fatal to the larvae of certain crop-eating pests.

Pests such as this corn earworm, *Helicoverpa zea*, are responsible for reduced yields in traditional corn crops.

NATURE OF SCIENCE

Scientists rarely agree 100% with each other, and sometimes they are very vehemently opposed to each other. In 1999 a group of researchers at Cornell University carried out a study in their laboratory to find out if the pollen from genetically modified Bt corn could have a negative effect on the larvae of the much-beloved monarch butterfly, a beautiful species admired for its impressive annual migrations from southern Canada and the USA down to Mexico for the winter. The study was immediately criticized by some members of the scientific community, who claimed that the quantities of transgenic pollen placed on the caterpillar's food was of a concentration that would not be possible in nature, and that more realistic experiments would need to be carried out in the field.

To find out more about this, use the hotlinks at the end of this section.

If you search the *Proceedings of the National Academy of Sciences of the United States of America* website, you should find many articles about the debate. See if you can find a paper by Karen S. Oberhauser's team: although there are many technical terms that you might not understand, the paper contains some graphs that you should be able to interpret concerning the overlap between when monarch butterflies are feeding on their favourite food, milkweed, and when corn is producing pollen. Which side of the debate is this scientist on?

TOK

The manipulation of genes raises some challenging questions. For many of these questions, there is not enough conclusive scientific data to reach a satisfactory answer.

- Is it ethically acceptable to alter an organism's genetic integrity?
- If the organism did not have that gene in the first place, could there be a good reason for its absence?
- Why are people so worried about this new technology? In selective breeding, thousands of genes are mixed and matched. With genetically modified organisms (GMOs), only one gene is changed. Is that not less risky and dangerous than artificial selection?
- Would strict vegetarians be able to eat a tomato that has a fish gene in it?
- Does research involving genetically modified (GM) animals add a whole new level to animal cruelty and suffering in laboratories?
- If Bt crops kill insects, what happens to the local ecosystem that relies on the insects for food or pollination?

Clones

Cutting, copying, and pasting genes

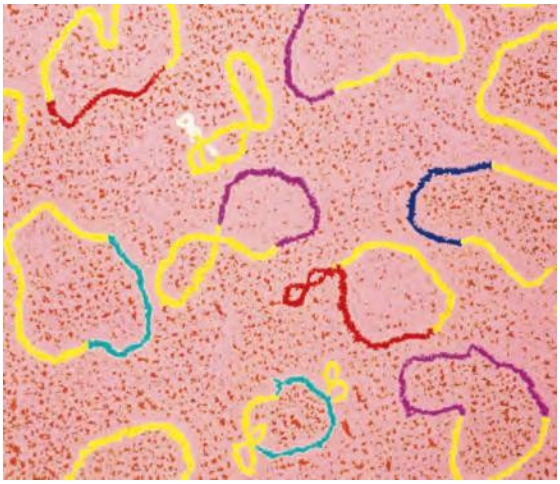
Although the laboratory techniques are complex, the concepts are not difficult.

Cutting and pasting DNA

The 'scissors' used for cutting base sequences are enzymes. Restriction enzymes called endonucleases find and recognize a specific sequence of base pairs along the DNA molecule. Some can locate target sequences that are sets of four base pairs, others locate sets of six pairs. The endonucleases cut the DNA at specified points. If both the beginning and the end of a gene are cut, the gene is released and can be removed from the donor organism. For pasting genes, the enzyme used is called DNA ligase. It recognizes the parts of the base sequences that are supposed to be linked together, called the sticky ends, and attaches them.

Copying DNA (DNA cloning)

Copying DNA is more complex, because a host cell is needed in addition to the cutting and pasting enzymes described above. Although yeast cells can be used as host cells, the most popular candidate in genetic engineering is the bacterium *Escherichia coli*.



This is a false-colour electron micrograph of plasmids.

Like other prokaryotes, most of the genetic information for *E. coli* is in the bacterium's single chromosome. However, some DNA is found in structures called plasmids. Plasmids are small circles of extra copies of DNA floating around inside the cell's cytoplasm. To copy a gene, it must be glued into a plasmid.

To do this, a plasmid is removed from the host cell and cut open using a restriction endonuclease. The gene to be copied is placed inside the open plasmid. This process is sometimes called gene splicing. The gene is pasted into the plasmid using DNA ligase. The plasmid is now called a recombinant plasmid and it can be used as a vector, a tool for introducing a new gene into an organism's genetic makeup.

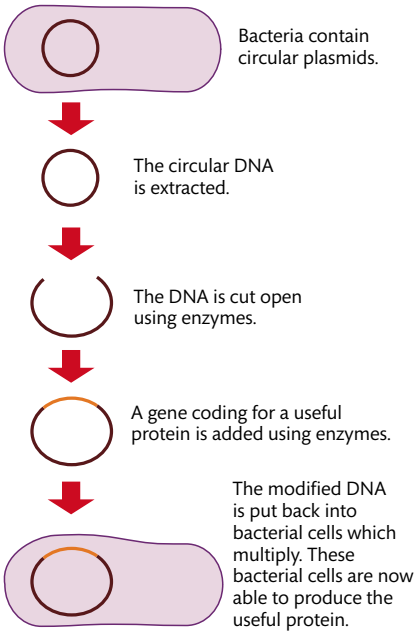


Figure 3.37 Gene splicing involves introducing a gene into a plasmid and it is one of the techniques used in genetic engineering to make a genetically modified organism.

In the final step needed for copying (or cloning) the gene, the vector is placed inside the host bacterium and the bacterium is given its ideal conditions in which to grow and proliferate. This is done by putting the bacterium into a bioreactor, a vat of nutritious liquid kept at a warm temperature.

Not only does the host cell make copies of the gene as it reproduces, but because the gene is now in its genetic makeup, the modified *E. coli* cell expresses the gene and synthesizes whatever protein the gene codes for. This process has been used successfully to get *E. coli* to make human insulin, a protein needed to treat diabetes (see Section 2.7). The older technique for obtaining insulin involves extracting it from cow and pig carcasses from the meat industry, but this has caused allergy problems. Using recombinant human DNA avoids that problem.

Genetically modified organisms

A genetically modified organism (GMO) is one that has had an artificial genetic change made using the techniques of genetic engineering, such as gene transfer or

recombinant DNA as described above. One of the main reasons for producing a GMO is so that it can be more competitive in food production. Another common reason is to 'teach' a bacterium to produce proteins that are useful in medical applications, as we saw with insulin.

Transgenic plants

The simplest kind of genetically modified (GM) food is one in which an undesirable gene has been removed. In some cases, another, more desirable, gene is put in its place, while in other cases only the introduction of a new gene is needed, no DNA has to be removed.

Whichever technique is applied, the end result is either that the organism no longer shows the undesired trait or that it shows a trait that genetic engineers want. The first commercial example of a GM food was the Flavr Savr tomato. It was first sold in the USA in 1994, and had been genetically modified to delay the ripening and rotting process so that it would stay fresher longer. Although it was an ingenious idea, the company lost so much money from the project that it was abandoned a few years later.

Another species of tomato was modified by a bioengineering company to make it more tolerant to higher levels of salt in the soil. This made it easier to grow in areas with high salinity. One of the claims of the biotech industry is that GM foods will help solve the problem of world hunger, by allowing farmers to grow foods in various, otherwise unsuitable, environments. Critics point out that the problem of hunger in the world is one of food distribution, not food production.

Another plant of potential interest to the developing world is a genetically modified rice plant that has been engineered to produce beta carotene in the rice grains. The aim is that the people who eat this rice will not be deficient in vitamin A (the body uses beta carotene to form vitamin A).

Transgenic animals

One way of genetically engineering an animal is to get it to produce a substance that can be used in medical treatments. Consider the problem faced by people with haemophilia. The reason their blood does not clot is because they lack a protein called factor IX. If such people could be supplied with factor IX, their problem would be solved. The least expensive way of producing large amounts of factor IX is to use transgenic sheep. If a gene that codes for the production of factor IX is associated with the genetic information for milk production in a female sheep, she will produce that protein in her milk.

In the future, a wide variety of genetic modifications may be possible, perhaps inserting genes to make animals more resistant to parasites, to make sheep produce pre-dyed wool of any chosen colour, to produce prize-winning show dogs, faster racehorses … The possibilities seem almost boundless, and it is difficult to imagine what the future might be like.

Natural methods of cloning

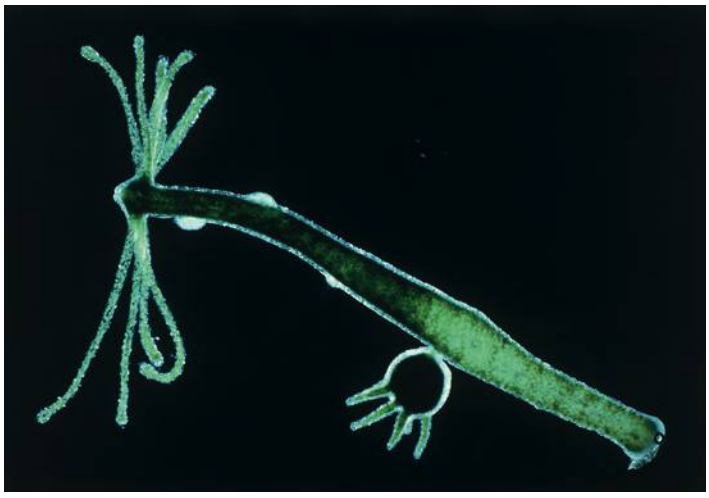
Nature invented cloning long before humans did. Certain plants, such as strawberry plants, can send out horizontal structures to allow a new strawberry plant to grow a short distance from the original plant. The new plant will be an exact genetic copy of

the first one, because only one parent was involved and no meiosis and fertilization was used to add variety to the genetic makeup of the plant.

It is possible to 'clone' a strawberry plant by asexual reproduction. The stems and leaves planted in the smaller pot will grow into a new plant.

If planted in the ground, a potato will grow into a new plant. The plant will be genetically identical to (will be a clone of) the original potato plant. This is an advantage for the plant, because there is no need to rely on pollen to fertilize the flowers, but it can be a disadvantage, because if all potato plants in a population are clones, it means that not only do they have the same good qualities, they also have the same weaknesses. If the population is attacked by a pathogen such as potato blight, it could wipe out the population. Historians will tell you of the dangers of this, notably in Ireland in the middle of the 19th century, when 1 million people died of starvation. Of course, historians will also tell you that there were other causes; history is complex, but the potato blight was a major factor in the famine.

What about animals: can they clone themselves the way plants sometimes do? Although this is extremely rare, and exceptional, among certain invertebrates, one animal that is capable of reproducing asexually by making clones of itself is the hydra, *Hydra vulgaris*. This freshwater organism is in the same phylum as sea jellies, sea anemones, and coral polyps. If food sources are plentiful, small buds will form on its body, develop into adults, and break off to form new, genetically identical, hydra. This process is called budding, and you may have observed this in electron micrographs of yeast cells. Similar to the plant examples (strawberries and potatoes), hydra are also capable of sexual reproduction.



A hydra is capable of natural cloning called budding.

Investigating the factors that affect the rooting of stem cuttings

Design an experiment to assess one factor affecting the rooting of stem cuttings. The basic idea is to cut a few centimetres of stem from a healthy plant and place it into an appropriate medium either sticking up or having it lying flat. Typical plants to try are impatiens, begonias, jade, or African violet.

Be sure to do some research to find a plant species that forms roots easily in either water or a solid medium. Take into account your geographical location and try to find plants that can be acquired locally and that will be in season in your area at the time you are carrying out the experiment.

Some possibilities to consider for your designed investigation are:

- the application of hormones such as ethylene, auxin, or gibberellins (be aware of the fact that certain types of auxins can be destroyed by light or by soil bacteria)
- abiotic factors such as light, temperature, and water (note that for light, not only could the intensity be changed, but the duration could be altered to simulate long days/short nights or short days/long nights)
- the medium in which the roots form, such as soil, sand, agar, or water
- the presence/absence of leaves on the stem
- horticultural techniques, such as wounding or girdling.

Once you carry out your experiment, any successful new plants that grow will be clones of the original plant.

There are ethical and legal considerations to consider: in certain circumstances, it is illegal to copy a plant in this way. Plants bought at a garden centre or nursery are often the result of many years of work on the part of horticulturalists and they can have intellectual property rights on their creative work. It could be argued that the purpose of your cloning exercise is educational and not for profit, but still, it is best to consider the intellectual property issues involved before choosing your plant.

Animals cloned from embryos

The definition of a clone is a group of genetically identical organisms, or a group of cells artificially derived from a single parent. In either case, the resulting cells or organisms were made using laboratory techniques. In farming, clones have been made for decades by regenerating plant material or by allowing an *in vitro* fertilized egg to divide to make copies of itself. When cloning happens naturally in animals (including humans), identical twins are produced.

The first evidence of an experimental attempt to make artificial clones was performed by Hans Dreisch in the 1890s with sea urchin embryos. He was able to separate cells from a single sea urchin embryo and grow two identical embryos. The aim of his experiment was not to create clones but, looking back, we can say that he serendipitously invented a new technique. Serendipity is a good concept to understand in science. It refers to an unexpected but positive discovery and happens when someone is looking for the answer to one question and accidentally finds the answer to a completely different question.

With the correct laboratory equipment, it is possible to separate cells from a growing embryo of an animal, and place the separated cells in the uterus of a female of that species and get artificial twins, triplets, quadruplets, etc., depending on how many cells were separated. Remember that embryonic cells are undifferentiated cells so there is nothing exceptionally astounding about this kind of cloning. Remember, nature has been doing this for a long time by forming identical twins.

Animals cloned from adult cells

Clones and cloning

Until recently, cloning was only possible using genetic information from a fertilized egg cell. After dividing many times, some of the cells will specialize into muscle cells, others into nerves, others into skin, and so on, until a foetus forms. For a long time, it was thought that once a cell has gone through differentiation, it cannot be used to make a clone. But then there was Dolly.

This is Dolly with Ian Wilmut, a member of her cloning team.

Cloning using a differentiated animal cell

In 1996, a sheep by the name of Dolly was born. She was the first clone whose genetic material did not originate from an egg cell. Here is how researchers at the Roslin Institute in Scotland produced Dolly (see Figure 3.38).

1. From the original donor sheep to be cloned, a somatic cell (non-gamete cell) from the udder was collected and cultured. The nucleus was removed from a cultured cell.
2. An unfertilized egg was collected from another sheep and its nucleus was removed.
3. Using an electrical current, the egg cell and the nucleus from the cultured somatic cell were fused together.
4. The new cell developed *in vitro* in a similar way to a zygote, and started to form an embryo.
5. The embryo was placed in the womb of a surrogate mother sheep.
6. The embryo developed normally.
7. Dolly was born, and was presented to the world as a clone of the original donor sheep.

This kind of cloning is called reproductive cloning because it makes an entire individual. The specific technique of reproductive cloning is called somatic cell nuclear transfer, because it uses a cell that is not an egg cell (therefore it is a somatic cell), and it has had its nucleus removed and replaced by another nucleus.

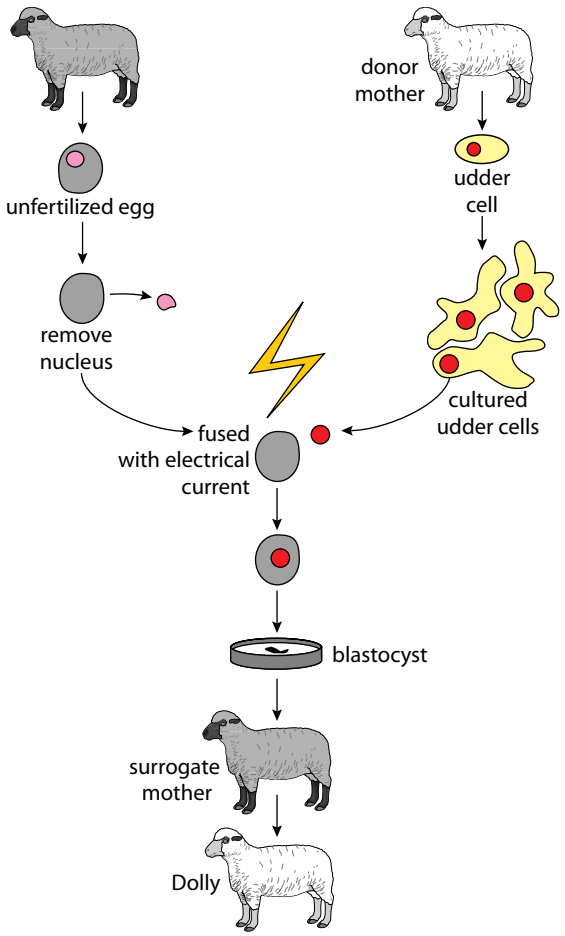


Figure 3.38 The step-by-step process of how the clone Dolly was made.

Cloning using undifferentiated cells

In some cases, scientists are not interested in making an organism but simply in making copies of cells. This second type of cloning is called therapeutic cloning, and its aim is to develop cells that have not yet gone through the process of differentiation. As the first technique in this area involved using embryos, the cells are referred to as embryonic stem cells, and the branch of laboratory work that investigates therapeutic cloning is called stem cell research.

Ethical issues surrounding therapeutic cloning

Because therapeutic cloning starts with the production of human embryos, it raises fundamental issues of right and wrong. Is it ethically acceptable to generate a new human embryo for the sole purpose of medical research? In nature, embryos are created only for reproduction, and many people believe that using them for experiments is unnatural and wrong.

However, the use of embryonic stem cells has led to major breakthroughs in the understanding of human biology. What was once pure fiction is coming closer and closer to becoming an everyday reality, thanks to stem cell research. Some of the aims of current research are to be able to grow:

- skin to repair a serious burn
- new heart muscle to repair an ailing heart
- new kidney tissue to rebuild a failing kidney.

With very rare exceptions, the vast majority of researchers and medical professionals are against the idea of reproductive cloning in humans. However, there is a growing popularity for therapeutic cloning because the potential of stem cell research is so enticing.

The idea of cloning often provokes strong negative reactions from people, especially when the only information they have comes from science fiction or horror films.

When making ethical decisions about what is good and bad, or right and wrong, it is important to be as well informed as possible.

In dealing with the ethical issues of cloning, it should be stressed that there are two distinct forms of cloning:

- reproductive cloning, making copies of entire organisms
- therapeutic cloning, making copies of embryonic stem cells.

Some people think that both are unacceptable, others think both are fine, and some are in favour of one but not the other. Where do you stand?

To learn more about gene transfer, go to the hotlinks site, search for the title or ISBN, and click on Chapter 3: Section 3.5.

Exercises

15 Explain why PCR is necessary.

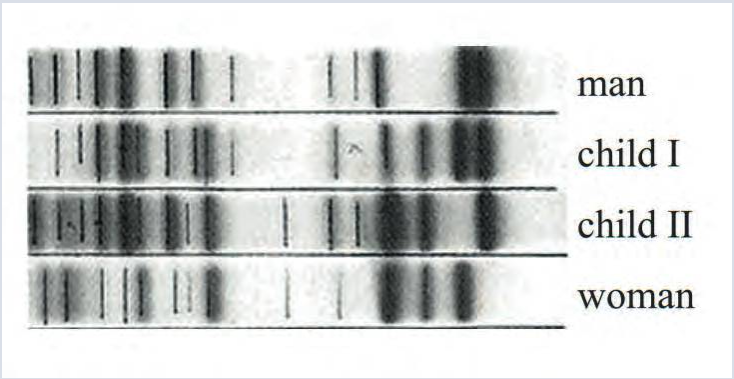
16 Explain the central ethical issue concerning stem cell research.

17 Justify whether the benefits outweigh the risks in genetically modifying plants and animals.

18 Look at the foods in your house. Are food labels today effective at indicating whether or not the food is genetically modified? Justify your answer.

Practice questions

1 What conclusion can be made from the following evidence from an analysis of DNA fragments?



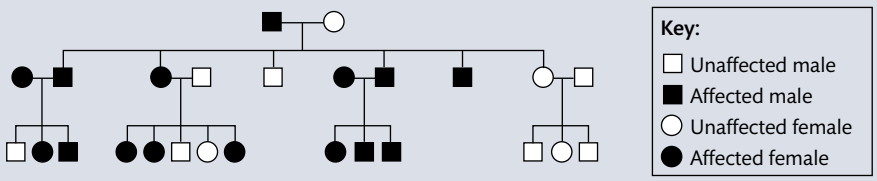
A Both children are related to both parents.

B Child I is related to the man but child II is not.

C Both children are unrelated to either of the parents.

D Child II is related to the man but child I is not. (*Total 1 mark*)

2 What evidence is given in the pedigree chart below to establish that the condition is caused by a dominant allele?



A Two unaffected parents have unaffected children.

B Two affected parents have affected children.

C An affected parent and an unaffected parent have affected children.

D Two affected parents have an unaffected child. (*Total 1 mark*)

3 Which of the following is an inherited disease that is due to a base substitution mutation in a gene?

A Trisomy 21

B Sickle cell anaemia

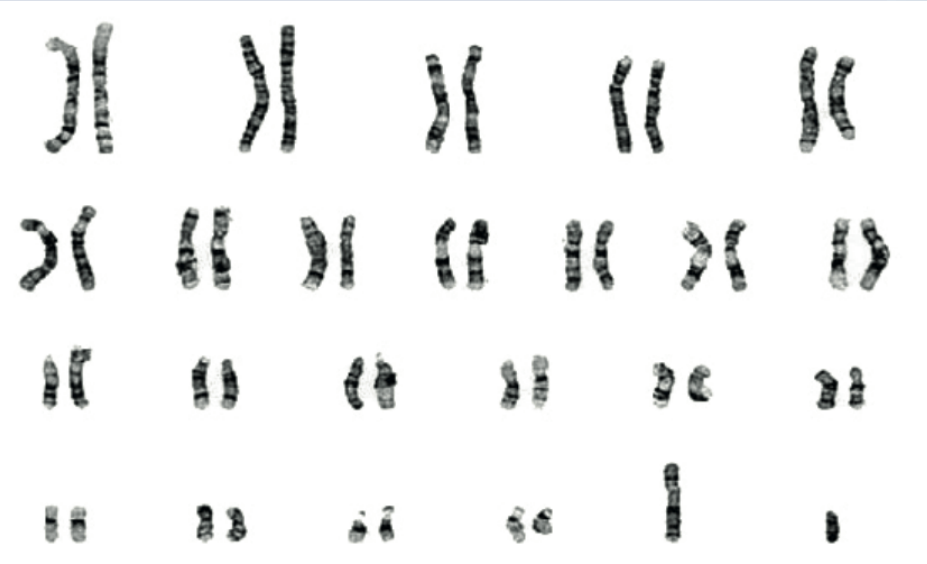
C AIDS

D Type II diabetes (*Total 1 mark*)

4 Outline some of the outcomes of the sequencing of the human genome. (*Total 3 marks*)

5 Describe the role of sex chromosomes in the control of gender and inheritance of haemophilia. (*Total 7 marks*)

6 What does the karyotype below correspond to?



A A normal male.

B A normal female.

C A female with Down syndrome.

D A male with Down syndrome. (*Total 1 mark*)

7 Describe the inheritance of ABO blood groups. (*Total 9 marks*)

8 Explain why carriers of sex-linked (X-linked) genes must be heterozygous. (*Total 2 marks*)

04 Ecology

Essential ideas

4.1 The continued survival of living organisms, including humans, depends on sustainable communities

4.2 Ecosystems require a continuous supply of energy to fuel life processes and to replace energy lost as heat.

4.3 Continued availability of carbon in ecosystems depends on carbon cycling.

4.4 Concentrations of gases in the atmosphere affect climates experienced at Earth's surface.

This colourful vegetation and blue sky are a stark contrast to what we would see on the surface of our inhospitable neighbours, the Moon, Mars, or Venus. Our planet shows complex interactions between the atmosphere, water, and living organisms.

Could you live on the Moon or on another planet such as Mars? If you had to make a list of what you would need, there would be some obvious things, such as liquid water, food, and oxygen gas to breathe. But is it possible to maintain life for a long period of time outside established ecosystems?

For example, if you brought bottles of oxygen gas with you to breathe, eventually they would run out. What could you bring with you that could supply oxygen regularly? The same questions can be asked about food and water.

A group of researchers tried such an experiment here on Earth by building a sealed living space called Biosphere II in the desert of Arizona, complete with a rainforest, a miniature ocean, land for growing food, and livestock to provide eggs and milk. A small group of people lived inside for 2 years in the early 1990s, and they learnt a great deal about sustaining life in a closed system. Such an experiment helps us to learn how we might set up a base on the Moon or perhaps Mars but, more importantly, it helped the people living inside appreciate what a delicate balance there is between air, water, and life: a balance that is complex and can be disrupted by actions with unintended consequences.

4.1 Species, communities, and ecosystems

Understandings:

- Species are groups of organisms that can potentially interbreed to produce fertile offspring.
- Members of a species may be reproductively isolated in separate populations.
- Species have either an autotrophic or heterotrophic method of nutrition (a few species have both methods).
- Consumers are heterotrophs that feed on living organisms by ingestion.
- Detritivores are heterotrophs that obtain organic nutrients from detritus by internal digestion.
- Saprotrophs are heterotrophs that obtain organic nutrients from dead organisms by external digestion.
- A community is formed by populations of different species living together and interacting with each other.
- A community forms an ecosystem by its interactions with the abiotic environment.
- Autotrophs obtain inorganic nutrients from the abiotic environment.
- The supply of inorganic nutrients is maintained by nutrient cycling.
- Ecosystems have the potential to be sustainable over long periods of time.

NATURE OF SCIENCE

Looking for patterns, trends, and discrepancies: plants and algae are mostly autotrophic but some are not.

Applications and skills:

- Skill: Classifying species as autotrophs, consumers, detritivores, or saprotrophs from a knowledge of their mode of nutrition.
- Skill: Setting up sealed mesocosms to try to establish sustainability.
- Skill: Testing for association between two species using the chi-squared test with data obtained by quadrat sampling.
- Skill: Recognizing and interpreting statistical significance.

Guidance

- *Mesocosms can be set up in open tanks, but sealed glass vessels are preferable because entry and exit of matter can be prevented but light can enter and heat can leave. Aquatic systems are likely to be more successful than terrestrial ones.*
- *To obtain data for the chi-squared test, an ecosystem should be chosen in which one or more factors affecting the distribution of the chosen species varies. Sampling should be based on random numbers. In each quadrat the presence or absence of the chosen species should be recorded.*

The interdependence of living organisms

In 1980 there was a major volcanic catastrophe at Mount Saint Helens on the west coast of the USA. After the massive eruption, little was left of the forest and rivers that had existed on and around the mountain. The blast from the eruption knocked over massive adult trees as if they were straws.

Forest fires and hot gases burned everything in sight. Volcanic ash rained down, smothering the destroyed forest and covering the carcasses of the animals that died there. Many species that could escape fled the area. Although thousands of people were evacuated, a few did die that day; some of those who died were photographers trying to get the photo of a lifetime.

Yet, within months of the eradication of the ecosystem, life was back. Seeds, dropped by birds or blown in by the wind, germinated in the fertile volcanic ash. Little by little, insects, then birds, then small mammals, moved in. Within a couple of decades, a grassland and shrub ecosystem had reappeared. Today, thousands of species flourish in what had been a desolate landscape.

These trees were knocked down by the Mount Saint Helens eruption in 1980. The ecosystems on the mountain were destroyed.

What is a species?

The definition of a species is a group of organisms that can interbreed and produce fertile offspring. Members of the same species have a common gene pool (i.e. a common genetic background).

Species is the basic unit for classifying organisms. It is one of those words everyone thinks they know, but it is not an easy concept. A species is made up of organisms that:

- have similar physiological and morphological characteristics that can be observed and measured
- have the ability to interbreed to produce fertile offspring
- are genetically distinct from other species
- have a common phylogeny (family tree).

This giant panda has characteristics that set its species apart; it cannot breed with other species that are not giant pandas.

There are challenges to this definition, however. Sometimes, members of separate but similar species mate and succeed in producing hybrid offspring. For example, a horse and a zebra, or a donkey and a zebra, can mate and produce offspring that are called zebroids. In these examples, the parents are both equines (they belong to the horse family, Equidae), so they are related, but they are certainly not the same species. They do not possess the same number of chromosomes, which is one of the reasons why the hybrid offspring produced are usually infertile.

Other challenges to our definition of species include the following.

- What about two populations that could potentially interbreed, but do not because they are living in different niches or are separated by a long distance?
- How should we classify populations that do not interbreed because they reproduce asexually? (The definition above is clearly aimed at sexually reproducing organisms, and cannot be applied to bacteria or archaeans.)
- What about infertile individuals? Does the fact that a couple cannot have a child exclude them from the species? What about the technique of *in vitro* fertilization? What challenges does that pose to the definition of species?

The answers to these questions are beyond the scope of this book and the IB programme. However, you should always think critically about any definition: at first glance it may appear to be straightforward, but on closer scrutiny it can be cause for debate.

All domestic dogs are the same species

Domesticated dogs are all the same species: *Canis familiaris*. In theory, that means that any two dogs from anywhere in the world can mate and have puppies that will grow up and be able to mate with any other dogs, and have more puppies.

Hybrids

To understand the idea of fertile offspring, think about what happens when two different but similar species mate and produce offspring. For example, a female horse and a male donkey can mate and produce a mule. However, mules cannot usually mate to make more mules. Because the offspring (the mules) are not fertile, no new species has been created. Instead, a mule is called an interspecific hybrid. When a male lion and a female tiger are crossed, a liger is the name of the hybrid formed.

Hybrids face several challenges to continue as a population. For one thing, the vast majority of animal and plant hybrids are infertile. Even if one generation of hybrids is produced, a second generation is highly unlikely. This presents a genetic barrier between species.

Some examples of animal hybrids are:

- female horse + male donkey = mule
- female horse + male zebra = zorse
- female tiger + male lion = liger.

A liger is a hybrid between a lion and a tiger, and is considerably larger than either parent animal.

Populations can become isolated

If a group from a species is separated from the rest of the species, it might find itself evolving in a different way compared with the rest of the population. For example, mice have inadvertently crossed oceans after going on board ships looking for food, and found themselves hundreds if not thousands of kilometres away from where their parent population lived, perhaps on an island far from any mainland. Two or more mice can mate and have litters of mice that then form a new population on the island. This new population is reproductively isolated from the original population of mice. Compared with the original population on the mainland, an island population of mice may end up with different frequencies of certain alleles for a trait such as fur colour, with the result that the mice in the island population only have black fur, while the mice in the original mainland population can have either brown or black fur.

As well as bodies of water, there are other ways in which populations of the same species can be isolated from each other, such as mountain ranges or deep canyons. There are tree snails in Hawaii, for example, that are present on one side of a volcanic mountain but not the other. But physical objects are not always responsible for separating populations of a species. Think of a group of birds that migrate: if some of those birds arrive early in the springtime and start nesting before the others arrive, the early birds' genes will be isolated from the birds that arrive and nest later. If some birds in a population develop a mating call that is different from the others, this could also potentially separate one population into two groups: one that likes the old call and one that likes the new call. Over time, this might lead to speciation: a new species is formed from an old one. You will find out more about isolation and speciation in Section 10.3.

Autotrophs and heterotrophs

A sheep eating grass is an example of a heterotroph (the sheep) feeding on an autotroph (the grass).

Autotrophs

Some organisms are capable of making their own organic molecules as a source of food. These organisms are called autotrophs, and they synthesize their organic molecules from simple inorganic substances. This process involves photosynthesis. In other words, autotrophs can take light energy from the Sun, combine it with inorganic substances, and obtain a source of chemical energy in the form of organic compounds. Because autotrophs make food that is often used by other organisms, they are called producers.

Examples of autotrophs include:

- cyanobacteria
- algae
- grass
- trees.

Heterotrophs

Heterotrophs cannot make their own food from inorganic matter, and must obtain organic molecules from other organisms. They get their chemical energy from autotrophs or other heterotrophs. Because heterotrophs rely on other organisms for food, they are called consumers. Heterotrophs ingest organic matter that is living or has been recently killed.

Examples of heterotrophs include:

- zooplankton
- fish
- sheep
- insects.

Consumers

Organisms that are not capable of synthesizing their own food from inorganic components of their environment need to get their nourishment by ingesting (eating) other organisms. For example, humans are heterotrophs: we cannot simply lie out in sunlight to get our food the way phytoplankton and plants can. We are consumers: we need to eat other living organisms, whether they are products of autotrophs, such as fruits and vegetables, or products of heterotrophs, such as meat, eggs, honey and dairy products. Consumers take the energy-rich carbon compounds, such as sugars,

CHALLENGE YOURSELF

1 From the photo, identify the following:
- **(a)** non-living inorganic components, both visible and non-visible (these are referred to as abiotic components)
- **(b)** living components, both visible and non-visible (living organisms are referred to as biotic components)
- **(c)** autotrophs present, both visible and non-visible
- **(d)** heterotrophs present, both visible and non-visible.

Can you identify the biotic and abiotic components in this photo?

proteins, and lipids, synthesized by other organisms in order to survive. The only component in our diet that we can synthesize, by exposure to sunlight, is vitamin D. There are precursors in human skin that absorb ultraviolet (UV) light waves and produce vitamin D. But in order to get all the other types of molecules needed to keep us healthy, we need to consume other living things.

The minotaur beetle, *Typhaeus typhoeus*, is a detritivore.

Detritivores

Some organisms eat non-living organic matter. Detritivores eat dead leaves, faeces, and carcasses. Earthworms, woodlice, and dung beetles are detritivores found in the soil community. Many, but not all, bottom feeders in rivers, lakes, and oceans are detritivores.

Saprotrophs

Organisms called saprotrophs live on or in non-living organic matter, secreting digestive enzymes and absorbing the products of digestion. Saprotrophs play an important role in the decay of dead organic materials. The fungi and bacteria that are saprotrophs

are also called decomposers, because their role is to break down waste material. A mushroom growing on a fallen tree is secreting enzymes into the dead tissue of the tree trunk, in order to break down the complex molecules within the tree tissue, and then the mushroom absorbs the simpler energy-rich carbon compounds that are released by the action of the enzymes. Slowly, over time, the tree trunk decomposes as the molecules inside the wood are liberated and reused.

Communities

A community is a group of populations living and interacting with each other in an area. Examples include the soil community in a forest and the fish community in a river.

In ecology, the term 'interacting' can mean one population feeding on another, or being eaten. It can mean that one species provides vital substances for another, as in the case of symbiotic bacteria, which help certain plants get nitrogen while the bacteria grow in the plant root nodules. It can also mean that one species gets protection from another, as in the case of aphids being protected by ants from attacks by predators. Interacting can mean that one species relies on another for its habitat, as is the case for parasites living on or inside the bodies of other animals.

Fungi are saprotrophs. Although edible mushrooms are found in the fruit and vegetable section of your local supermarket, they are not classified by biologists as plants. It is arguable that they should be with other consumers in the meat section.

CHALLENGE YOURSELF

2 From Figure 4.1, pick three organisms and determine how many other organisms each one depends on. Which organisms depend on them? What about environmental factors? Which ones does each organism contribute to, and which ones does each depend on?

Figure 4.1 A tropical rainforest contains many interactions between living organisms and their environment.

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Classification is all about looking for patterns and grouping organisms together according to those patterns. This can be challenging when looking at algae. Because they are green and photosynthesize, organisms such as seaweed used to be considered to be plants, until closer examination revealed that they do not possess the structures we expect to find in plants, such as roots and leaves. Today seaweed is classified as algae. Classifying all algae as autotrophs is also a challenge, because some species live as parasites and do not actually use their photosynthetic capabilities.

An example of an easily identifiable plant to use for the fieldwork lab; in this case the plant is yarrow.

Ecosystems

The term abiotic refers to components of the environment that are non-living, such as water, air, and rocks. When abiotic measurements are taken of an environment, they can include temperature, pH, light levels, and the relative humidity of the air. Such things are often measured using electronic probes and data-logging techniques. Although these factors are not living entities, they are often of great interest to biologists because of the interactions that living things have with them. Temperature and humidity, for example, have a large influence on the types of plant life found in a community; an open marsh will not have the same kinds of plants as a dense forest. To find out to what extent a particular abiotic factor influences a species' distribution, many measurements must be taken, of both the abiotic and biotic (living) aspects of the environment. One technique used to determine the frequency and distribution of a species is random sampling.

Fieldwork

To understand random sampling, try this lab using quadrats. A quadrat is a square of a particular dimension that can be made of a rigid material such as metal, plastic, or wood. In this example, each group will use a 1-m^2 quadrat. Other materials you will need are: a table of random numbers from 1 to 99, a pencil, and something on which to record your data.

- Pick a well-defined area, such as a fenced-in pasture, public park, or a sports field with natural grass (be sure you have permission to work there first).
- Choose a species of plant that grows there that is easy to identify and that is widespread throughout the area, but not so numerous that counting the number of individuals growing in a square metre would take more than a minute or two. Possible examples are dandelions, docks, and yarrow, but the choice will depend on where you live and when you carry out the lab.
- Each group should start in a different part of the area and spin a pencil to determine a random direction. Then, with your group, look at the first number on the random number table and walk in the designated direction that number of steps. If the border of the area is reached before the designated number of steps has been taken, you should 'bounce' off the border like a ray of light off a mirror, and continue in the direction dictated by an angle of incidence that is the same as the angle of reflection.
- Place the quadrat down on the ground at the point determined by the number of steps, and decide which of the four sides will be the 'top' and 'right' of the quadrat.
- Identify and count the number of individuals of the chosen species found inside the borders of the quadrat at that position. If it is zero, record the result as such. Any plants touching the top or right should be considered 'in' and should be counted. Any plants touching the bottom or left side of the quadrat should be considered 'out' and not counted.
- Repeat this as many times as possible in, say, an hour: the more quadrats, the better. However, a typical sports field might be 5000 m^2, so there is no way a group can sample all 5000 m^2 and cover the entire field: that is why random sampling is used.
- Before leaving the area you are working in, measure its dimensions so that its total surface area can be determined. This might be challenging for an irregularly shaped pasture or park, in which case online aerial views of the area might be useful. In that case, note the scale of the image.
- Now you will carry out some data processing. Determine how many plants you hit per square metre, then use the surface area calculation to estimate the total number of individuals of that plant that are living in that area: (plants per m^2) × (surface area in m^2) = (population estimation).

If this experiment is done for two species of plants, are there any calculations you could do to compare the two? See the Mathematics, and information and communication chapter for more about statistical tests.

Alternative: if weather or space forces a group to do this indoors, the activity can be simulated with disks of paper or sticky notes scattered around a gymnasium. In such a case, use a smaller quadrat, maybe one that is 50 cm^2. An advantage of this alternative lab is that the person scattering the disks or sticky notes knows how many there are in total, and it is interesting to see how close the groups' estimations are to the known number.

Systematic sampling techniques

There is another way of using a quadrat rather than the random sampling described in the lab above: systematic sampling using a transect. A transect is a line traced from one environment to another, such as from a grassland into a woodland, or from an ocean's intertidal zone over dunes. The line might be 10, 25, or 50 m long, and can be made using a long tape measure or piece of string or rope. This method involves laying down a quadrat either every metre along the transect, or at specific intervals along the transect, for example every 2 m, 5 m, or 10 m, and then counting the organisms that hit each quadrat and then counting the organisms found within each quadrat. Notice how, unlike the example of the quadrats used in the fieldwork lab, there are no random numbers. Because the distances are measured carefully, this is especially interesting in cases where we want to see if there is a relationship between the distribution of organisms that live along the transect and an abiotic factor that changes along the transect, such as temperature, humidity, and light levels.

Worked example

Let's suppose a group of students has been working in a forest in late summer and they have measured several abiotic factors, including light intensity in two distinct areas: a heavily wooded area and an open prairie. Not surprisingly, they recorded major differences in light intensity between one area and the next. They identified a particular species of fern that grows in both areas but appears to prefer shaded areas compared with areas exposed to direct sunlight. After collecting their data, they wanted to see if the presence of ferns was statistically significantly larger in the shaded areas (the woodland) compared with the areas in direct sunlight (the prairie). The group was divided into two teams: one for the woodland and the other for the prairie. They used random sampling with 1-m^2 quadrats to get their data. If they found the fern growing in their quadrat, they recorded a 1, if not, a 0. Table 4.1 shows what their data looked like after 20 quadrats had been thrown by each group.

Quadrat	Shade (woodland)	Sunlight (prairie)	Quadrat	Shade (woodland)	Sunlight (prairie)
1	0	1	11	1	0
2	1	1	12	1	0
3	1	0	13	0	0
4	1	0	14	1	1
5	1	0	15	0	0
6	0	1	16	1	1
7	1	0	17	1	1
8	1	1	18	1	0
9	0	0	19	0	0
10	1	0	20	1	0

Table 4.1 Quadrat data for a fern species. Legend: 1=presence of ferns in quadrat, 0=absence of ferns in quadrat

Apply the chi-squared test to these data to decide whether the shade had an influence or not on the distribution of the fern. (See page 797 for an explanation of what the chi-squared test is, how it works, and what the values for the degrees of freedom should be.)

1 State the null hypothesis in this calculation.
2 Determine the number of degrees of freedom in this calculation.
3 Determine the critical value in order to obtain a 95% certainty that there is a statistically significant difference between these two sets of numbers.
4 Calculate the chi-squared value for these data.
5 Interpret this value. Does it mean we can accept or reject the null hypothesis?
6 Is there a statistically significant difference between these two sets of data?
7 Are there enough data to be confident of the results?

Solutions

1 The null hypothesis is 'the two categories (presence of fern and presence of shade) are independent of each other'. In other words, the distribution of this fern species is not related to shade.
2 Because there are two possible outcomes (fern present or fern not present), the number of degrees of freedom is 2 – 1 = 1.
3 According to the chi-squared table (see Table 5 on page 796), the critical value in order to obtain a 95% certainty is 3.84. This value is found under the column 0.05, which corresponds to a 95% certainty, and it is found in the row that has the degree of freedom of 1.
4 The chi-squared value is calculated to be 4.91. This is obtained using the following values in the contingency tables.

Table 4.2 The table of observed values

	Shade (woodland)	Sun (prairie)	Grand total
Fern absent	6	13	19
Fern present	14	7	21
Grand total	20	20	40

Below, the expected value of 9.5 is from the calculation: (20 × 19) ÷ 40 and the expected value of 10.5 is from the calculation (20 × 21) ÷ 40.

Table 4.3 The table of expected values

	Shade (woodland)	Sun (prairie)	Grand total
Fern absent	9.5	9.5	19
Fern present	10.5	10.5	21
Grand total	20	20	40

See page 797 of the Mathematics, and information and communication chapter for help with this calculation.

5 Because 4.91 is greater than the critical value of 3.84, this means we can rej ect the null hypothesis.
6 Yes, the two categories are related to each other. We can be 95% sure that there is a relationship between the fern distribution and amount of sunlight. In other words, it would be very unlikely that they are independent of each other.
7 Twenty quadrats sounds a bit small. In a random sample, there is always the chance that the sampling is not representative of the zone studied. If the zone in the sunlight was the size of a sports field, for example, it would have a surface area of approximately 5000 m^2. Twenty 1-m^2 quadrats represents 20 m^2 of that surface, meaning that only 0.4% of the field was actually sampled. The same can be said for the shaded area in the woods.

You can be asked about the chi-square test in IB biology exams. Be sure you know when the chi-square test can be used, the steps of how to do it, and how to interpret the results.

Where do autotrophs get their nutrients?

Unlike consumers, who need to eat organic food from plants and animals, autotrophs can make the food they need from their inorganic surroundings. Photosynthetic organisms, such as phytoplankton, cyanobacteria, and plants, are able to produce food by using carbon dioxide, water, and sunlight. They make food from air using sunlight energy. It is a truly remarkable process, and no consumers could survive on this planet without the initial production of food by autotrophs. Because of this ability to make food from inorganic substances, autotrophs are referred to as producers, and they are the start of food chains, which will be explored later in Section 4.2.

Long before plants evolved on Earth, cyanobacteria were photosynthesizing. Cyanobacteria have been producers for many ecosystems.

Nutrient cycling

When organisms such as trees need minerals to grow and stay healthy, where do they get them from? Even though tonnes of space dust fall on Earth each year, there is not enough to meet the mineral needs of all the organisms in the biosphere. As a result, ecosystems must recycle the carbon, nitrogen, and other elements and compounds necessary for life to exist. For this, organisms must find what they need within the materials available in their own habitat. The problem is that organisms absorb valuable minerals and organic compounds and use them to build their cells. These resources are then locked up and unavailable to others, except, of course, through feeding and decomposition.

Decomposers

An effective way to unlock the precious nutrients stored in the cells of plants and animals is through decay. Decomposers (saprotrophs and detritivores) break down the body parts of dead organisms. The digestive enzymes of decomposers convert the organic matter into a more usable form for themselves and for other organisms. For example, proteins from a dead organism are broken down into ammonia (NH_3) and, in turn, ammonia can have its nitrogen converted into useful nitrates (NO_3^-) by bacteria.

In this way, decomposers recycle nutrients so that they are available to other organisms and are not locked inside the bodies or waste products of organisms in the ecosystem. Decomposers play a major role in the formation of soil, without which plant growth would be greatly impaired, if not impossible. The rich black layer of soil called humus is made up of organic debris and nutrients released by decomposers. In a vegetable garden, a compost pile is used to convert plant waste from the garden and kitchen into rich humus that can then be used to grow new vegetables. The organisms doing the work inside the compost pile are decomposers.

Earth has various systems that interact.

Biosphere = where all living things are found.

Atmosphere = where all the gases in the air are found.

Lithosphere = where all the rocks are found.

Hydrosphere = where all the water is found.

Each one of these systems is closely linked with the others, and some, such as the biosphere, cannot exist in their current form without the other three.

When talking about the health of Planet Earth, we often use words like 'pollution' and 'waste'. Ecologists studying ecosystems noticed very quickly that what one organism considers to be waste is what another organism considers to be a valuable resource. For example, the nitrogen compounds found in rotting flesh or animal excrement are extremely useful for plant growth. In other words, 'pollution' can be considered to be simply misplaced resources. If we can figure out a system that makes waste useful, then it will be a sustainable system, and all the waste will be put to a new use as part of a cycle. If we cannot find a use for the waste we produce, then we truly are polluting our environment.

CHALLENGE YOURSELF

3 For each organism below, identify which type of nutrition is used: heterotroph; heterotroph that is a saprotroph; heterotroph that is a detritivore; autotroph.
- **(a)** The bacterium *Rhizobium*.
- **(b)** Fungi on a dead log.
- **(c)** Cyanobacteria floating where there is sunlight.
- **(d)** A snail scraping algae off a rock.

Rhizobium lives in the root nodules of legumes and fixes atmospheric nitrogen. These bacteria are symbiotic and receive carbohydrates and a favourable environment from their host plant.

Fungi on a dead log.

The sustainability of ecosystems

Thanks to this recycling of nutrients, ecosystems can continue to be productive and successful for long periods of time. The producers take simple inorganic compounds, such as carbon dioxide (CO_2), from their environment and convert them into energy-rich sugars, such as glucose ($C_6H_{12}O_6$). Those simple sugars can then be transformed into complex carbohydrates to make cellulose, to build up plant cell walls. Other nutrients can be added to form complex organic molecules such as lipids and proteins.

Consumers will then come along and eat the producers, and digest the complex organic compounds into simpler building blocks, such as amino acids and sugars, for growth and energy. When those consumers die, their cells and tissues are broken down by decomposers, and the minerals are returned to the soil. Producers can once again absorb the nutrients from the soil, and grow new sources of food. The cycle is complete. This process is sometimes informally referred to as the circle of life, but the more scientific term is nutrient cycling.

One example of nutrient cycling is the nitrogen cycle. Nitrogen is extremely important to living organisms, as it is one of the elements needed in nucleotides and amino acids, the building blocks of life. Without this element, organisms would not be able to make DNA or proteins, and thus life would be impossible. Nitrogen starts the cycle in gas form in the atmosphere, as N_2. Plants and animals are incapable of using nitrogen gas but some bacteria are able to transform it into useful forms, such as nitrates, in a process called nitrogen fixation. These usable nitrates are absorbed by plant roots (which is why some plants host the nitrogen-fixing bacteria in their root nodules, as seen in the Challenge yourself photo above), and so the plants pass on nitrogen-rich nutrients when they are consumed by animals. Both plants and animals return the nitrogen to the soil in a variety of ways. Urine and faeces, for example, contain

nitrogen compounds, which is why farmers often put animal manure on the fields where they grow crops. When plants and animals die, the nitrogen compounds are returned to the ground by decomposition. Going back to the question at the start of the chapter, about what you would bring to the Moon to live there, would you have listed decomposers? What about nitrogen-fixing bacteria?

A miniature world in a plastic bottle

You can set up your own ecosystem of microbes in a plastic bottle at home or in the lab. This is a long-term project, so make sure you have somewhere that you can leave the bottle for many weeks or months. The experiment you're going to set up is called a Winogradsky column.

For this lab, you will need some dark, wet mud. If you get it near a pond, be sure to take some pond water separately as well. Make sure you have permission to take the samples.

You will need an empty, clean transparent plastic drinks bottle that will hold 2 or 3 litres, mud (nice, slimy, stinky, mud works the best), shredded newspaper, crushed egg shells and raw egg yolk.

- Place the last three ingredients in about half a litre of mud, removing any large pieces from the mud such as sticks and stones. Mix the ingredients up and pour them into the bottom of the bottle. A funnel might be necessary. Add more mud on top of that until the bottle is two-thirds full. Add some water, but be sure to leave about 5 cm of air at the top of the bottle. Be sure there are no air bubbles in the mud.
- Place the cap on the bottle and leave it for many weeks near a sunny window: the micro-organisms in the bottle need sunlight. It is a good idea to take a photo at the beginning of the experiment, and then one every week or so. These photos will show you the changes that occur over time. Make sure you carefully label your experiment so that no one throws it away thinking it is rubbish.

Variations on the experiment include using: a glass container that can be sealed at the top; different types of mud; different sources of carbon dioxide instead of the egg shells; different sources of sulfur instead of the raw egg yolk.

To interpret the results you will have to do a bit of research to find out what the different colours mean. Each one represents a different kind of bacterial colony in the mud, and each of those transforms molecules for the others to use. As long as there is light entering the system, the column will continue to maintain a healthy microbial ecosystem for many months.

If you have the materials, the time, and the ambition, it is also possible to set up a more complex aquatic ecosystem that is hermetically sealed. Go to the hotlinks for suggestions of web sites that explain how to do this. Otherwise, do a search for keywords such as 'make your own ecosphere' or 'sealed terrarium'.

Ethical considerations: in accordance with the IB policy on the use of living organisms in experiments, it is best to avoid putting sentient beings in your ecosphere or mesocosm. Before adding snails or shrimp, for example, you would need to decide if you can justify exposing such organisms to things they would not encounter in their natural habitat such as low oxygen levels or low food supplies. Fish, tadpoles, or invertebrates bigger than a few millimetres are probably not appropriate.

Two major events in the modern environmental movement were the first photographs of Planet Earth from space, during the Apollo missions in the late 1960s and early 1970s, and the publication in 1962 of *Silent Spring* by Rachel Carson, a book imagining a future with no more birds.

In the decades since those events, more and more people have become concerned about our ability as a species to have enough space, water, and food for everyone. Ecologists who study human interactions with the other forms of life on Earth, and interactions with the non-living components of the environment, are concerned about our future, and think that international cooperation is necessary to solve complex global issues such as insufficient drinking water supplies, overfishing, global climate change, loss of forests and topsoil, bleaching of coral reefs, and the depletion of countless other natural resources. Their plea is that we need to adopt international policies to limit human impact and maintain sustainable practices.

Governments and societies are going to need to think about what is necessary for this to happen. Several paths could be explored, such as better education, international agreements and policies, higher taxation of unsustainable activities, or discussions of population control to limit human impacts. All of these have advantages and disadvantages and would need to be debated. One phrase that often comes up when looking for ways in which we can help our planet is 'act locally, think globally'. This could be a possible topic for discussion in your biology class or TOK class.

To learn more about micro-ecosystems setting up sealed mesocosms, go to the hotlinks site, search for the title or ISBN, and click on Chapter 4: Section 4.1.

Exercises

1. Distinguish between habitat and ecosystem.
2. Explain why decomposers are so important in nature.
3. Humans can be considered to be omnivores, meaning they eat autotrophs and heterotrophs. Give two examples of autotrophs in the human diet and two examples of heterotrophs in the human diet.

NATURE OF SCIENCE

Use theories to explain natural phenomena: the concept of energy flow explains the limited length of food chains.

4.2 Energy flow

Understandings:

- Most ecosystems rely on a supply of energy from sunlight.
- Light energy is converted to chemical energy in carbon compounds by photosynthesis.
- Chemical energy in carbon compounds flows through food chains by means of feeding.
- Energy released from carbon compounds by respiration is used in living organisms and converted to heat.
- Living organisms cannot convert heat to other forms of energy.
- Heat is lost from ecosystems.
- Energy losses between trophic levels restrict the length of food chains and the biomass of higher trophic levels.

Applications and skills:

- Skill: Quantitative representations of energy flow using pyramids of energy.

Guidance

- *Pyramids of number and biomass are not required. Students should be clear that biomass in terrestrial ecosystems diminishes with energy along food chains, due to loss of carbon dioxide, water, and other waste products, such as urea.*
- *Pyramids of energy should be drawn to scale and should be stepped, not triangular. The terms producer, first consumer, and second consumer, and so on should be used, rather than first trophic level, second trophic level, and so on.*
- *The distinction between energy flow in ecosystems and cycling of inorganic nutrients should be stressed. Students should understand that there is a continuous but variable supply of energy in the form of sunlight but that the supply of nutrients in an ecosystem is finite and limited.*

The importance of sunlight to ecosystems

The best studied ecosystems are those found on Earth's surface, whether they are on land or in surface water. Such systems rely on sunlight, and they will be the main focus of this section. Be aware, however, that there are other, less well-studied, ecosystems that exist in total darkness, such as those in deep ocean water and those found deep underground, but these are not well understood because they are so difficult to access.

All life that you see around you on Earth's surface relies either directly or indirectly on sunlight. If a person eats an omelette for breakfast, for example, the eggs were made indirectly with energy from sunlight. How? The hen that laid the eggs probably ate some kind of grain in order to get the energy to make the eggs, and the plant material eaten by that hen was from a producer, and the producer used sunlight to transform carbon dioxide and water into energy-rich carbon compounds. Take away the sunlight from this scenario, and the eggs could not have been produced because the hen would not have had any grains to eat.

Sunlight is the initial source of energy for all vegetation.

The role of photosynthesis

As seen in Section 4.1, photosynthetic organisms such as phytoplankton and plants take simple inorganic carbon dioxide, CO_2, and convert it into energy-rich sugar, $C_6H_{12}O_6$. The addition of minerals allows the producers to synthesize complex molecules such as cellulose, proteins, and lipids. Notice what is happening in this process: light energy from the Sun is being converted into chemical energy (food). Chemical energy refers to the fact that organic compounds, such as carbohydrates, proteins, and lipids, are rich in energy, thanks to the chemical bonds that exist between the carbon atoms and other atoms. This is what makes fruits, grains, and vegetables good food sources. Consumers cannot 'eat' sunlight and air, but they can eat carbohydrates, proteins, and lipids. The chemical energy in these organic compounds can be measured in calories or kilocalories, which we see listed on food packaging. One way to release the chemical energy from organic compounds is to digest the food, another way is to burn it. Burning wood in a fire is a good example of turning chemical energy in the organic compounds of the wood into light energy (and heat energy).

Food chains

By feeding on producers, consumers can utilize the chemical energy to grow and stay healthy. For example, a cow (the consumer) grazing in a field of grass (the producer) is taking chemical energy from the grass and digesting the organic compounds to help build meat or milk inside its own body. Humans can consume the meat or milk from the cow to benefit from the chemical energy the cow has obtained from the grass. Such a pattern of feeding is called a food chain. The process of passing energy from one organism to another through feeding is referred to as the flow of energy through a food chain.

When studying feeding habits, it is convenient to write down which organism eats which by using an arrow. Thus, herring → seal indicates that the seal eats the herring. When the seal's eating habits are investigated and the herring's diet is considered, new organisms can be added to the chain: copepods (a common form of zooplankton) are eaten by the herring, and great white sharks eat seals. Lining up organisms with arrows between them is how food chains are represented. Here are three examples of food chains from three different ecosystems.

Grassland ecosystem:

grass → grasshoppers → toads → snakes → hawk

River ecosystem:

algae → mayfly larvae → juvenile trout → kingfisher

Marine ecosystem:

diatoms → copepods → herring → seals → great white shark

The definition of a food chain is a sequence showing the feeding relationships and energy flow between species. In other words, it answers the question 'What eats what?' The direction of the arrow shows the direction of the energy flow.

Biologists use the term trophic level to indicate how many organisms the energy has flowed through.

The first trophic level is occupied by the autotrophs or producers. The next trophic level is occupied by the primary consumers (organisms that eat the producers), and the trophic level after that is occupied by secondary consumers (organisms that eat primary consumers).

Three trophic levels can be seen in this photograph: a producer, a primary consumer, and a secondary consumer.

Cellular respiration and heat

In the example of the grass and the grasshoppers, inside a grasshopper chemical energy is used for cellular respiration. Glucose originally produced by the grass is converted by the grasshopper's cells into carbon dioxide and water. This chemical reaction generates a small amount of heat in each of the grasshopper's cells. Any heat

generated by cellular respiration is lost to the environment. Although this might be more obvious in mammals, which can give off considerable amounts of heat, even grasshoppers will lose heat to the environment. If the grasshopper is eaten, some of the chemical energy in its body (in the form of protein, for example) is passed on to the next organism (a toad, for example). If the grasshopper dies and is not eaten, detritivores and decomposers will use its available energy.

The cells of decomposers also carry out cellular respiration and, as a result, any heat produced this way will also be lost to the environment. This is just one source of energy loss from one trophic level to the next, as we will see.

How many trophic levels are shown in this Peruvian scene?

Heat cannot be recycled

This section has mentioned heat being lost, but what does it mean when heat is 'lost'? As you may already know from other science courses, there is a law about energy stating that energy cannot be created or destroyed, only converted from one form to another. We have seen that light energy can be converted into chemical energy by the process of photosynthesis. We have also seen that during the process of cell respiration, not all the energy is converted into useful energy (ATP) by the cell: some of it is converted to heat energy. Although this keeps mammals warm, once the heat leaves an organism's body, it cannot be used again as a biological energy resource. So, for the organism, this energy is 'lost'. It has not disappeared, however; it has simply been converted into a form that the organism can no longer use as a source of energy.

Where does the heat go?

Because ecosystems are made up of lots of respiring organisms, each losing heat, heat is lost from the ecosystem. Once the heat has radiated into the surrounding environment, the ecosystem cannot take back that heat to use it. Notice how this is very different from nutrient cycling with substances such as nitrogen and carbon. Unlike nutrients, energy cannot be recycled. It is passed from one trophic level to the next, and when it leaves the ecosystem it is not reusable. Is this a problem? Usually no, because the Sun is constantly providing new energy to producers. The energy is converted to chemical energy and passed on from one trophic level to the next. However, if, for some reason, the Sun stops shining, because it is blocked from Earth by clouds or particles in the sky (as happens after large volcanic eruptions), then the food chain is affected.

Only chemical energy can be used by the next trophic level (see Figure 4.2), and only a small amount of the energy that an organism absorbs is converted into chemical energy. In addition, no organism can use 100% of the energy present in the organic molecules of the food it eats. Typically, only 10–20% of the energy available is used from the previous step in a food chain. This means that as much as 90% is lost at each level.

Here are the main reasons why not all of the energy present in an organism can be used by another organism in the next trophic level.

The most popular theory for the mass extinction that wiped out the dinosaurs (and many other organisms) at the end of the Cretaceous period, is that the Sun's energy was blocked by particles in the air after a large object smashed into Earth. The darkened skies meant that producers could not get enough sunlight to continue making enough food to feed the consumers.

- Not all of an organism is swallowed as a food source, some parts are rejected and will decay.
- Not all of the food swallowed can be absorbed and used in the body, for example owls cough up the hair and bones of the animals they eat, and undigested seeds can be found in the faeces of fruit-eating animals.
- Some organisms die without having been eaten by an organism from the next trophic level.
- There is considerable heat loss as a result of cellular respiration at all trophic levels (shown by the wavy arrows in Figure 4.2), although the loss of heat varies from one type of organism to the next. Most animals have to move, which requires much more energy than a stationary plant needs. Warm-blooded animals need to use a considerable amount of energy to maintain their body temperature.

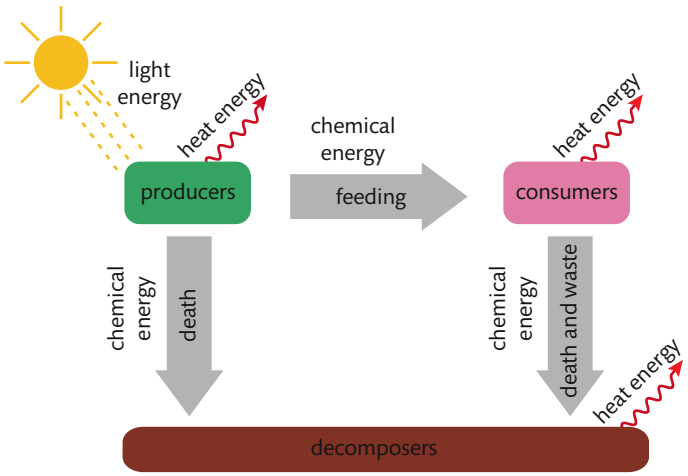

Figure 4.2 Energy flow and energy loss.

Whereas nutrients are constantly cycled in ecosystems, energy is not. The cycles of growth, death, and decomposition show how nature recycles nutrients, but energy pyramids show that energy flows through a system and is lost. This is why new energy must arrive in the form of sunlight in order to keep the system going.

Pyramid of energy

A pyramid of energy is used to show how much and how fast energy flows from one trophic level to the next in a community (see Figure 4.3). The units used are energy per unit area per unit time: kilojoules per square metre per year (kJ m^{-2} yr^{-1}). Because time is part of the unit, energy pyramids take into account the rate of energy production, not just the quantity.

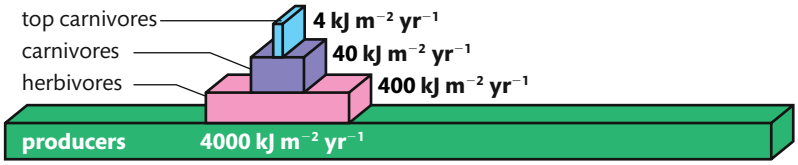

Figure 4.3 Pyramid of energy.

Because energy is lost, each level is always smaller than the one before. It would be impossible to have a higher trophic level wider than a lower trophic level, for example, because organisms cannot create energy, they can only transfer it inefficiently.

Be careful not to confuse pyramids of energy with pyramids of numbers: pyramids of numbers show the population sizes of each trophic level, not the energy.

Worked example

Using the following information, construct a pyramid of energy. Try your best to make it to scale.

In an ecosystem, the producers make 10 000 kJ m^{-2} yr^{-1}. 1000 kJ m^{-2} yr^{-1} of energy is passed on to the herbivores. In the third trophic level, the carnivores absorb 100 kJ m^{-2} yr^{-1} and the top predators who eat them get 10 kJ m^{-2} yr^{-1}.

Solutions

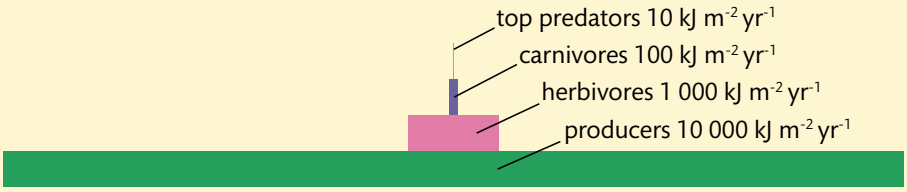

Figure 4.4 A pyramid of energy showing a 90% loss of energy at each trophic level.

Food webs and energy levels in trophic levels

If you look back at the three examples of food chains earlier in this chapter, you will notice that they are all either four or five organisms long. Although some food chains can have up to six trophic levels, most have four. The number of levels is limited by how much energy enters the ecosystem. Because so much is lost at each level, low energy at the start will quickly be lost, whereas abundant energy at the start can sustain several trophic levels. So the number of organisms in the chain as well as the quantity of light available at the beginning will determine how long the chain is.

The biomass of a trophic level is an estimate of the mass of all the organisms within that level. It is expressed in units of mass, but also takes into account area or volume. For example, in terrestrial ecosystems, fields of wheat might produce 1 tonne $acre^{-1}$ yr^{-1} in one area of the world, whereas another area might produce 3 tonnes $acre^{-1}$ yr^{-1}. Although there may be other factors, the amount of sunlight reaching the fields will influence the biomass, so that sunnier parts of the world can produce more wheat. In contrast, cooler climates or ones with fewer hours of sunlight per year have a lower biomass and therefore cannot support as many organisms. The fields of wheat could be the start of a food chain that consists of field mice, snakes, and hawks. Some molecules along the food chain cannot participate in the accumulating biomass because they are lost in various forms: carbon dioxide is lost from the organisms during cellular respiration, water is lost during transpiration and evaporation from the skin, and waste products including urea are excreted. So, just as not all energy gets passed on from one trophic level to the next, not all biomass gets passed on either.

Look at the food chains in Figure 4.5 showing a river ecosystem. Notice how the trophic levels link together into a food web. Sometimes it is necessary to describe a food web, rather than a food chain, because an organism such as a juvenile trout eats not only caddis fly larvae but also the larvae of other species. Notice that the trophic levels are labelled with the letter T, and think about the biomass in each: did you ever wonder how scientists estimate the total biomass in each trophic level? That question

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The statements about what limits the length of a food chain and prevents one from going beyond a certain number of trophic levels can be explained by the energy pyramids shown earlier. Because so much energy is lost at each level (90%), the only way to have more energy available for the top level is to increase the energy going into the bottom level, for the producers. As the energy collected by producers is limited by the amount of sunlight reaching Earth's surface, it is difficult to increase it.

goes beyond the scope of this section, but, if you are interested, use the hotlinks at the end of this section to find more information.

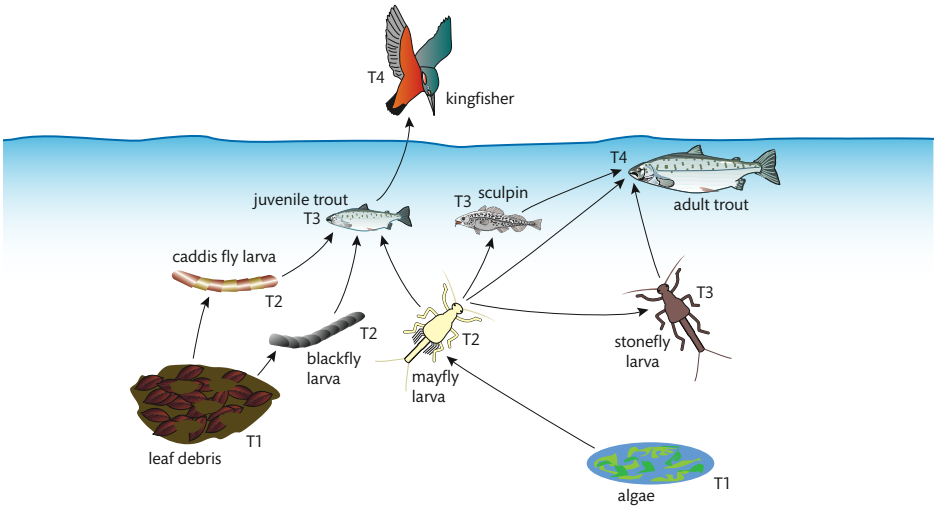

Figure 4.5 A food web from a river ecosystem showing trophic (T) levels.

To learn more about biomass, go to the hotlinks site, search for the title or ISBN, and click on Chapter 4: Section 4.2.

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Ever since Thomas Malthus predicted in the late 18th century that humans would eventually run out of food, scientists and researchers have wondered how big the human population can get before the amount of biomass available as food becomes insufficient to feed everyone. Techniques of food production have changed dramatically since Malthus' time, and we have not yet reached the tipping point he predicted. The industrialization of agriculture, as well as the invention of artificial fertilizers, brought about the Green Revolution, allowing farmers to produce many times more biomass than ever before on the same farms. Today, in some countries, tonnes of grain sit and rot in silos, while in other countries people go hungry. Are questions of world hunger simply questions of technology and biomass production? Do we need to produce more food to feed the hungry? Will the Malthusian catastrophe eventually come about: will we run out of food for our species some day? Or should we be confident that countries will work together to find the best solution? What questions do scientists still need to answer in order to guide policy makers, and how will they answer those questions?

Exercises

4 Look at these food chains again. Name the trophic levels (as producer or consumer) for each organism listed.

(a) Grassland ecosystem:
grass → grasshoppers → toads → snakes → hawk

(b) River ecosystem:
algae → mayfly larvae → juvenile trout → kingfisher

(c) Marine ecosystem:
diatoms → copepods → herring → seals → great white shark

5 From the following information, construct a food web:

- grass is eaten by rabbits, grasshoppers, and mice
- rabbits are eaten by hawks
- grasshoppers are eaten by toads, mice, and garter snakes
- mice are eaten by hawks
- toads are eaten by hognose snakes
- hognose snakes are eaten by hawks
- garter snakes are eaten by hawks.

6 From the food web you have drawn, what is the trophic level of the toad?

4.3 Carbon cycling

Understandings:

- Autotrophs convert carbon dioxide into carbohydrates and other carbon compounds.
- In aquatic ecosystems carbon is present as dissolved carbon dioxide and hydrogen carbonate ions.
- Carbon dioxide diffuses from the atmosphere or water into autotrophs.
- Carbon dioxide is produced by respiration and diffuses out of organisms into water or the atmosphere.
- Methane is produced from organic matter in anaerobic conditions by methanogenic archaeans and some diffuses into the atmosphere or accumulates in the ground.
- Methane is oxidized to carbon dioxide and water in the atmosphere.
- Peat forms when organic matter is not fully decomposed because of acidic and/or anaerobic conditions in waterlogged soils.
- Partially decomposed organic matter from past geological eras was converted either into coal or into oil and gas which accumulates in porous rocks.
- Carbon dioxide is produced by the combustion of biomass and fossilized organic matter.
- Animals such as reef-building corals and molluscs have hard parts that are composed of calcium carbonate and can become fossilized in limestone.

Applications and skills:

- Application: Estimation of carbon fluxes due to processes in the carbon cycle.
- Application: Analysis of data from air monitoring stations to explain annual fluctuations.
- Skill: Construct a diagram of the carbon cycle.

Guidance

- *Carbon fluxes should be measured in gigatonnes.*

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Making accurate, quantitative measurements: it is important to obtain reliable data on the concentration of carbon dioxide and methane in the atmosphere.

Carbon

As seen in Chapter 2, the element carbon is the cornerstone of life as we know it. Carbon is such a crucial element to living organisms that it is part of the definition of a living thing. You will recall that the term 'organic' implies that carbon is present. Hence, life on Earth is referred to as carbon-based life.

Not only is carbon found in the biosphere in organic molecules such as carbohydrates, proteins, lipids, and vitamins, it is also found in the atmosphere as carbon dioxide and in the lithosphere as carbonates and fossil fuels in rocks. The biosphere refers to all the places where life is found, and the lithosphere refers to all the places where rocks are found. Petroleum, from which products such as gasoline, kerosene, and plastics are made, is rich in carbon because it originated from partially decomposed organisms that died millions of years ago.

As seen in Figure 4.6, carbon is constantly being cycled between living organisms and inorganic processes that allow the carbon to be available. The carbon atoms that make up the cells of the flesh and blood of the giraffe, for example, came from the vegetation the giraffe ate. Eating organic material provides newly dividing cells in the giraffe's body with a fresh supply of carbon-based energy-rich molecules with which the cells can carry out work. When cellular respiration is complete, carbon dioxide is released into the atmosphere, and when the giraffe dies, its body will be eaten by scavengers and the remains broken down by decomposers. Some of the carbon from the giraffe's body will go back into the atmosphere as carbon dioxide when the decomposers perform cellular respiration. This section will look at some of the many different forms carbon can take as it is cycled by nature.

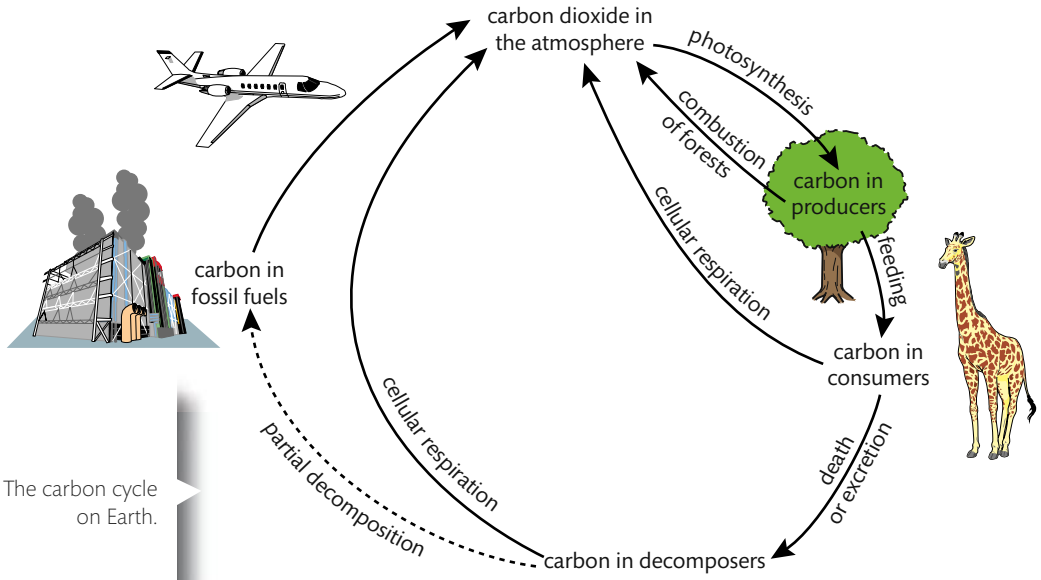


Figure 4.6 The carbon cycle on Earth.

The role of autotrophs in the carbon cycle

Let's start with food. Photosynthetic autotrophs take carbon dioxide from the atmosphere and convert it into carbohydrates. Here is the unbalanced chemical equation for photosynthesis.

	inorganic carbon dioxide goes in		an organic carbohydrate (glucose) comes out	
H_2O +	CO_2	→	$C_6H_{12}O_6$	+ O_2

Figure 4.7 The unbalanced equation for photosynthesis.

The sugar on the right-hand side of the equation (in green) is a source of food, not only to the autotroph synthesizing it, but also to the organisms that feed on the autotrophs. In its inorganic form on the left, as atmospheric carbon dioxide (in blue), the carbon is not usable as a food source by the autotrophs or by any consumers. Few people fully realize how dependent the biosphere is on energy from the Sun for food production. And the biosphere includes us.

From the $C_6H_{12}O_6$ molecules, autotrophs can manufacture other compounds. Fructose and galactose are other sugars that can be made by plants from glucose. Connecting the sugars together into a long chain can make starch; plants can store energy for a future season or a future generation in the form of starch granules, tubers, or seeds. Plants and algae need to build their cell walls with cellulose, which is also made from long chains of glucose. Glucose is the starting point for making other organic compounds that are not carbohydrates, such as lipids and amino acids. These compounds are necessary to make useful things such as cell membranes and proteins such as enzymes. To synthesize these non-carbohydrates, other elements such as nitrogen must be added to the glucose.

Do you use cellulose in your life? You are probably wearing cellulose right now, because any textiles made of cotton are made of plant cellulose. Books are printed on cellulose, because paper pulp is from plant material. And if you use a car or a bus that runs on biofuel, that vehicle is being powered by cellulose.

Carbon in aquatic ecosystems

As you know from drinking fizzy drinks or carbonated water, carbon dioxide can dissolve in water. Although the oceans, lakes, and rivers of the world are not as fizzy

as a carbonated drink, they contain dissolved carbon dioxide because carbon dioxide from the atmosphere can be absorbed by the water. Remember also that organisms living in water produce carbon dioxide through cellular respiration. As the carbon dioxide is dissolved in the water, it forms an acid. The pH of water decreases as the amount of carbon dioxide increases. This is why carbonated water has an acidic taste.

$$CO_2 + H_2O \rightarrow H_2CO_3 \text{ (carbonic acid)}$$

$$H_2CO_3 \rightarrow H^+ + HCO_3^- \text{ (hydrogen carbonate ion)}$$

When dissolved in water, the carbonic acid forms the H^+ in the equation above, which is an ion that can influence pH. But what is of interest to us is the hydrogen carbonate ion, HCO_3^-, because this is a good example of an inorganic carbon-based molecule that participates in the carbon cycle.

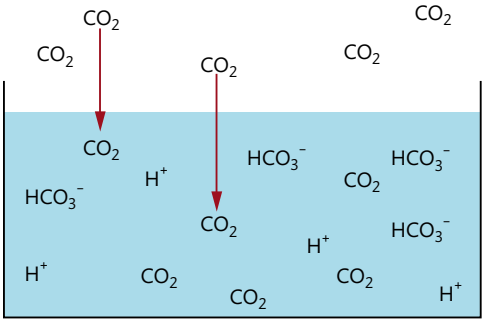


Figure 4.8 The forms of carbon available in aquatic ecosystems: dissolved carbon dioxide and hydrogen carbonate ions.

Cycling of carbon dioxide

Carbon dioxide is absorbed by photosynthetic autotrophs such as photosynthetic bacteria, phytoplankton, plants, and trees. As you will recall, these producers are eaten by consumers, which use the carbon in their bodies. Cellular respiration from all trophic levels, including decomposers, produces carbon dioxide, which is released back into the environment. This carbon dioxide diffuses into the atmosphere or into the water, depending on whether the organism is terrestrial or aquatic.

Carbon is available to photosynthetic organisms as carbon dioxide gas in the air or dissolved in water. It is available to consumers in the form of carbohydrates, proteins, and lipids, but can also be absorbed in the form of ions such as carbonate ions.

Methane in the carbon cycle

Other carbon compounds are produced by microbes such as archaeans. Members of the Archaea include methanogens, which are anaerobic (they live in environments with no oxygen). When these methanogenic archaea metabolize food, they produce methane (CH_4) as a waste gas. You should be familiar with methane because it is the same gas used in laboratories (the flame of Bunsen burners) and in homes for cooking and heating.

These microbes are also common in wetlands, where they produce marsh gas, which can sometimes glow mysteriously at night, but they are also responsible for producing methane gas in the digestive tracts of mammals, including humans. With large herds of cattle being raised worldwide, there is a concern that the quantities of methane they produce are contributing to the greenhouse effect, which will be discussed in the next section.

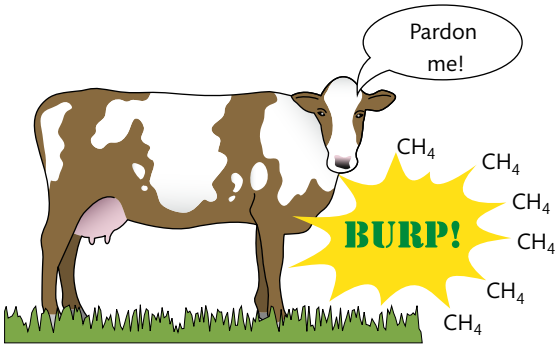


Figure 4.9 Methane gas production.

In her description of living in Biosphere II, a hermetically sealed experimental facility developed in Arizona, USA, researcher Jane Poynter said she had a new appreciation of the air she was breathing. In an interview on the TED Radio Hour in 2013 she said, 'The most profound experience I had in the biosphere was the experience of not only being completely dependent on my biosphere, but being absolutely a part of my biosphere in a very literal way. I mean, as I walked through the biosphere, I was incredibly conscious of the fact that the plants surrounding me were providing me with the oxygen that I needed to breathe, and that I was providing them some of the carbon dioxide they needed to grow.' She says we need to think about this when we are living in Biosphere I, which, in case you hadn't worked it out, is Planet Earth.

The oxidation of methane

How does the burning of fossil fuels produce carbon dioxide? Look at the chemical reaction below, showing methane burning in oxygen gas:

$$CH_4 + 2O_2 \rightarrow 2H_2O + CO_2$$

Methane is the main ingredient in the fossil fuel we call natural gas. As you can see from the formula, this chemical reaction involves oxygen gas from Earth's atmosphere. When the methane is oxidized, the two molecules produced are water vapour and carbon dioxide gas.

The carbon found in the molecule CH_4 was borrowed from a CO_2 molecule that was removed from the atmosphere millions of years ago during photosynthesis. It then took the methane gas millions of years to form and accumulate underground. When we burn natural gas provided by the petroleum industry, we return that carbon to the atmosphere in the form of carbon dioxide. Normally, we would think that this is just part of a balanced cycle. The problem is, one part of the cycle takes millions of years and the other part, the burning of fossil fuels, is very rapid.

Peat as a fossil fuel

Another organic substance that can be used as a fossil fuel is partially decomposed plant material called peat. Peat is a kind of waterlogged soil found in certain types of wetlands, such as mires and bogs, which can be found in the British Isles, Scandinavia, northern Russia, some eastern European countries, northern Canada, northern China, the Amazon River basin, Argentina, northern USA (notably Alaska) and parts of Southeast Asia. Peat is very dark in colour and only certain types of vegetation can grow on its surface, such as sphagnum moss. Although peat is a heterogeneous mixture of many things, at least 30% of its dry mass must be composed of dead organic material for it to be called peat. The soil that forms peat is called a histosol, and a layer of peat is typically between 10 and 40 cm thick.

Slabs of peat left to dry in Scotland.

Walking on peatlands can be a bit of a challenge because they are very spongy. The high levels of water on peatland force out the air that would normally be between the soil particles. As a result, anaerobic conditions are created, which allows certain types of microorganisms to grow but prevents the growth of microorganisms that would

normally help in the decomposition of plant material. Hence many of the energy-rich molecules that would have been fed upon by decomposers are left behind and transformed, over thousands of years, into in energy-rich peat.

Another characteristic of peatlands is the pH of the waterlogged histosol: it is very acidic. Just as with low oxygen levels, if the acidity is not conducive to the decomposers, they will not be able to do their work. High acidity contributes to the fact that non-decomposed material accumulates. In the pools of acidic water that can be found on these wetlands, certain types of organisms can be found that are not found anywhere else, such as some species of aquatic beetles.

In order for it to be usable as a fuel, cut peat is dried out to reduce its high levels of humidity. It is cut into slabs, granules, or blocks, and moved to where it is needed. Like all fossil fuels, however, peat takes a very long time to form and is not considered to be a renewable source of energy. Once all the peat in a wetland has been harvested it is gone; it is unrealistic to wait for new peat to form, so new sources of fuel are needed.

In economic periods when oil prices are high, peat can be a competitive energy source, but when oil prices are low this is not the case, and there have been decades during which many countries decided to drain their wetlands to replace them with forests and farmland. In some cases, environmental concerns about the preservation of wetlands, because they are an important part of the ecosystem and a habitat for unique species, have prevented the digging and drainage of peatlands. Another reason to preserve wetlands is that pollen trapped in deep layers of the bogs thousands of years ago can provide evidence of what the climate was like in the past, giving us 'libraries' of biotic information.

Oil and gas as fossil fuels

In some cases, when left in the correct conditions, partially decomposed peat can be further transformed into coal. Over millions of years, sediments can accumulate above the peat, and the weight and pressure of those sediments compresses the peat. Under conditions ideal for the formation of coal, the sedimentation continues until the carbon-rich deposits are not only under huge pressure but also exposed to high temperatures because they have been pushed far below Earth's surface. The pressure and heat cause chemical transformations associated with lithification, which is the transformation of sediments into solid rock. During lithification, the molecules are compacted and rearranged. What is of great interest to industries using coal is the hydrocarbons, the long chains of carbon atoms attached to hydrogen atoms (see Figure 4.10).

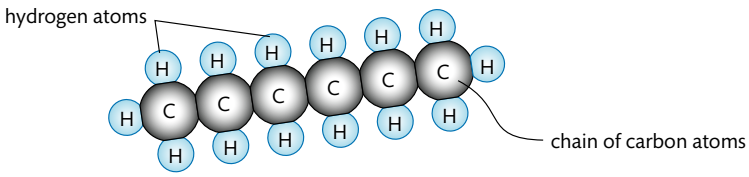


Figure 4.10 A hydrocarbon chain.

The C–H bonds hold a significant amount of energy, and, because there are many of them in long chains, each hydrocarbon molecule is rich in energy ready to be released by burning.

In order to use coal for energy, it must be extracted from below the ground, which is why mining is necessary. Coal is found in seams, where the layers of sediments were deposited, covered, and then transformed and often twisted and deformed by geological forces over millions of years.

A lump of coal.

In addition to coal, chemical transformations underground can produce other petroleum products, such as crude oil and, as we have seen, natural gas.

For this, we have to go far back in time, before dinosaurs roamed Earth. During the Carboniferous period, hundreds of millions of years ago, some places in the world that are now dry land were underwater and hosted abundant aquatic or marine life, including algae and zooplankton. For example, the dry deserts of Saudi Arabia used to be under the Tethys Ocean, back when all the continents were still stuck together in the supercontinent called Pangaea.

At that distant time in Earth's past, under conditions ideal for the formation of petroleum products, the dead remains of the organisms in the water did not fully decompose at the bottom of the ocean, and instead formed layers of sediment along with silt. In conditions lacking oxygen (anoxic conditions), the decaying material started to form sludge, as some parts of the organisms' cells decayed while others did not. One component of dead algae and zooplankton that is not easily broken down is the lipid component of their cells. Accumulated lipids that are trapped in sediments at the bottom of an ocean form a waxy substance called kerogen. It, too, is rich in hydrocarbons and, like the formation of other fossil fuels, is transformed by pressure and heat as sediments accumulate above it and cause its molecules to rearrange.

The natural production of kerogen is a long process, and the right conditions have only occurred in certain parts of the world. Figure 4.11 shows some of the places where crude oil has been found in the world.

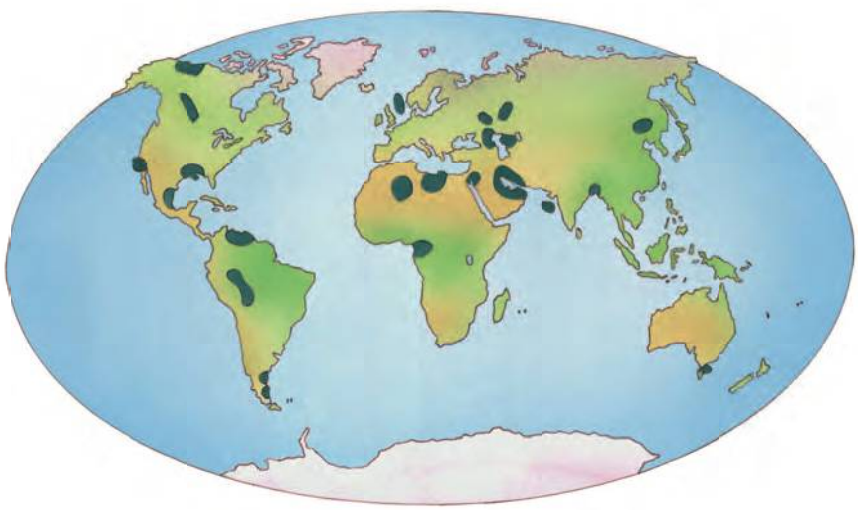
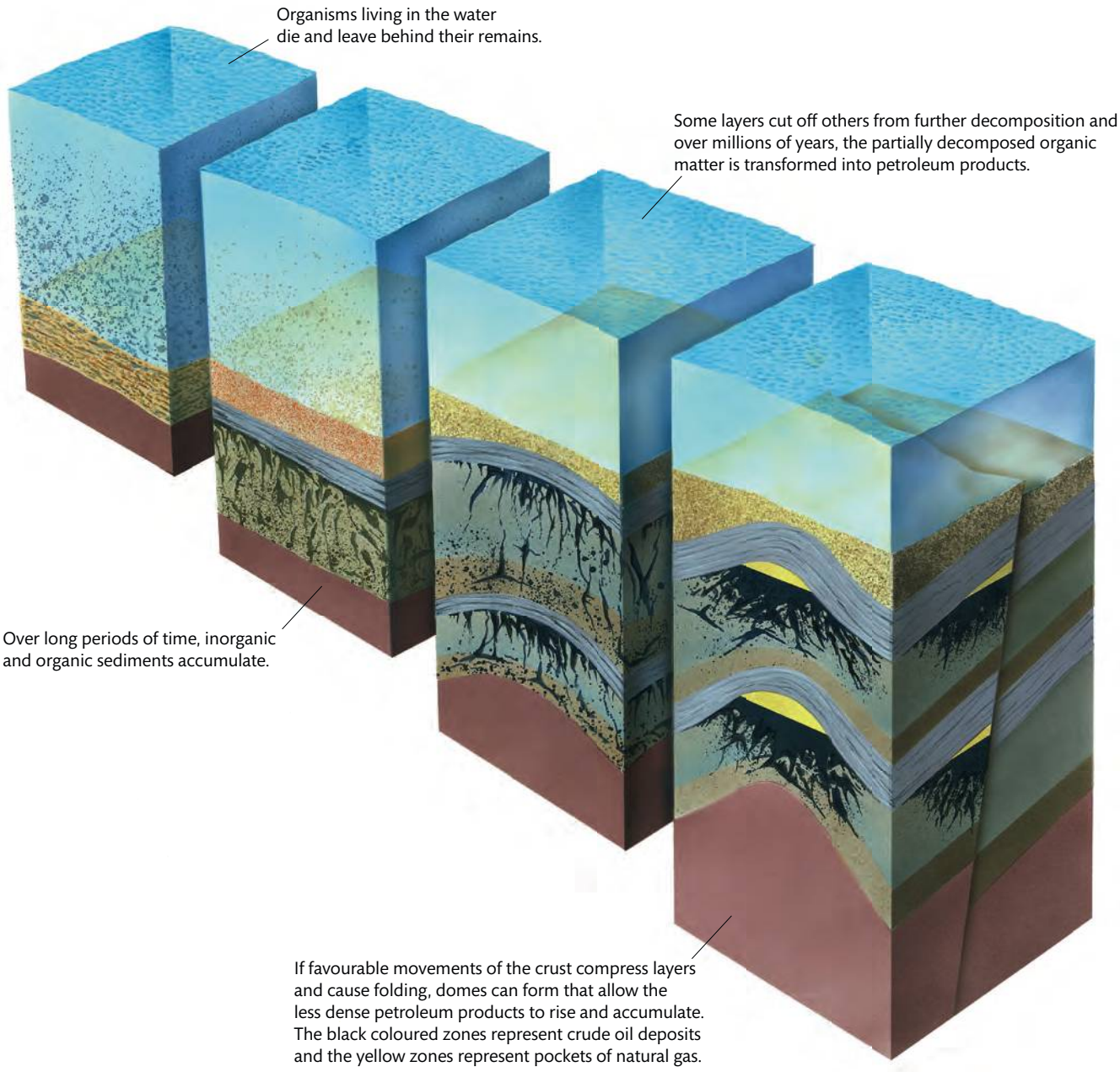


Figure 4.11 World deposits of crude oil.

Over millions of years, and after geological transformation, the kerogen in porous sedimentary rock becomes crude oil or, if it is in a gas state, natural gas. Both of these petroleum products are less dense than rock, so they tend to rise through cracks in the rocks towards the surface.

Figure 4.12 Formation of gas and oil.



In order to be used by humans, petroleum products must be trapped and pooled under a non-porous rock, preferably one that is bent by tectonic movement into a dome, as seen in Figure 4.12. This kind of formation allows large quantities of useful gas and oil to collect together in a productive reservoir. Geologists study the porosity and deformations of rock layers in order to determine which parts of the world might contain exploitable gas and oil reserves.

The term 'fossil fuel' refers to the fact that the source of energy in the fuel comes from partially decayed once-living organisms that died long ago, often millions or hundreds of millions of years ago. Because they take so long to form, fossil fuels are considered to be a non-renewable resource.

When an oil field is discovered and crude oil is pumped out of the ground, it is sent to refineries to be separated into the various products that we use every day, using an apparatus called a fractionating tower. Such an apparatus allows the heaviest, most dense molecules with the longest hydrocarbon chains to accumulate at the bottom, and the lightest, least dense molecules with the shortest hydrocarbon chains to accumulate at the top. Look at Figure 4.13 and see how many of these petroleum products you rely on every day. One fraction that is not shown in the diagram is naphtha, which might not sound familiar but it is the main ingredient used to make plastics.

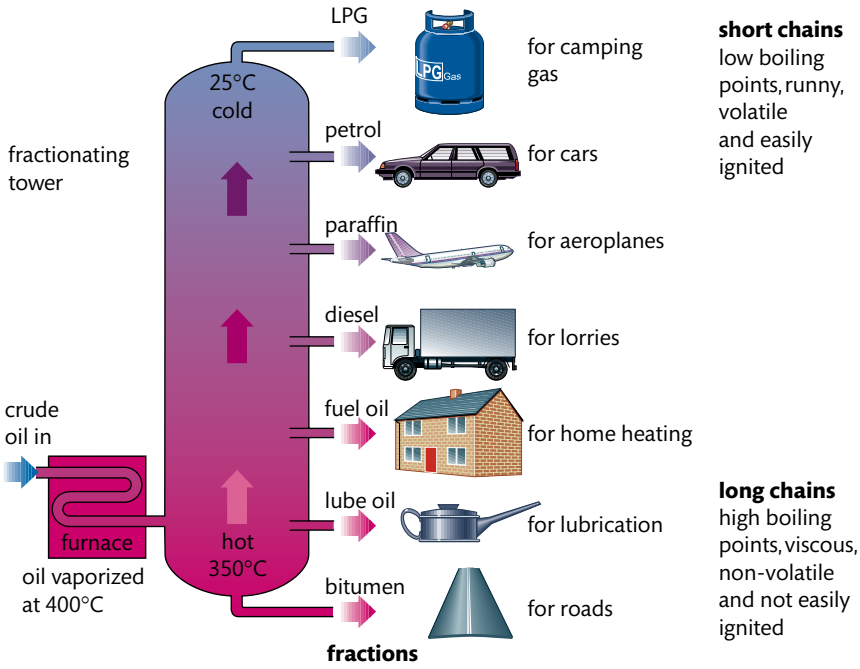


Figure 4.13 The many uses of petroleum products in our everyday lives. How many of these products do you rely on every day? One fraction that is not shown in the diagram is naphtha, which is the main ingredient used to make plastics.

Crude oil has the nickname 'black gold'. Oil and gas companies are prepared to go to the most inaccessible places in the world to dig out the black gold, whether it is in the hot sands of deserts or at the bottom of icy cold oceans. And it is worth all that trouble. In 2012, more than a third of the 50 companies with the highest revenues worldwide were oil and gas companies, four of which had revenues exceeding $400 000 000. Such a number is difficult to grasp, but it is higher than the gross domestic product (GDP) of most countries in the world.

Carbon dioxide is produced when fossil fuels are used

Just as we saw with methane previously, substances rich in hydrocarbons can be oxidized using oxygen gas from the atmosphere when they are burned. If you have ever made a fire on a beach or at a campsite, you know that organic material such as wood is capable of releasing a considerable amount of energy in the form of light and heat. Wood is not the only fuel of biological origin that can be burned: many people living in non-industrialized areas of the world use biomass in the form of animal dung as a source of energy. The dried dung of domesticated animals such as cows can be burned and used for various purposes, including cooking. Fresh, wet dung can be mixed with other refuse from a farm and put into a large container, where methane-producing microorganisms will decompose and ferment the material to produce flammable methane gas, as seen in Figure 4.14. Unlike fossil fuels, biofuels made in a biogas generator like this do not take millions of years to form.

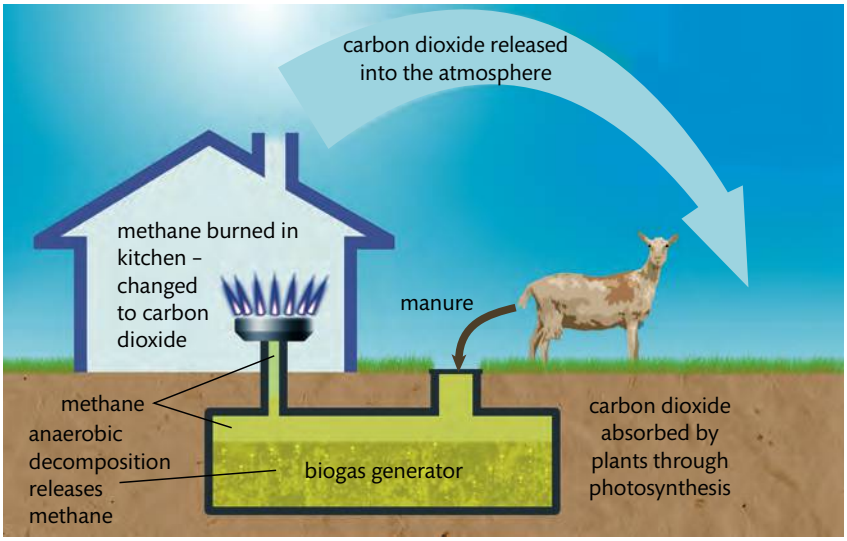


Figure 4.14 Biogas production and use.

In an effort to reduce fossil fuel consumption, some countries, such as the USA and Brazil, have introduced biofuel programmes using ethanol made from crops such as corn and soybeans. The plant material is fed to microorganisms that ferment it and in the process release ethanol. The ethanol is added to gasoline for vehicles, and contributes to a reduction in gasoline use. Standard vehicles cannot use more than 25% ethanol and need 75% or more gasoline (this mix can also be called gasohol), but vehicles specially adapted for biofuels can run solely on ethanol.

Using a different technique, biodiesel can be made from vegetable oils or animal fat. Some people have even modified their cars so that they run on the waste oil from deep-fat fryers at fast food restaurants.

Although it can be argued that using biofuels allows countries to reduce their dependence on imported fossil fuels, the burning of any biomass still releases carbon dioxide into the atmosphere. The difference is that, unlike fossil fuels, the carbon dioxide from biofuels was removed from the atmosphere by plants just a few months or years before the biofuel was used.

Some farmers in the world are growing crops that are not destined for human food nor for animal feed but rather for fuel to power cars and city buses. Brazil and the USA have been innovators in this practice, and it is a way of cycling carbon that depletes fewer fossil fuel reserves. There is a down side to it, however: some questions arise about the morality of such a practice. In the countries where this policy has been put in place, there are people starving. Is it acceptable to use food crops as fuel for motor vehicles instead of making it available for humans to eat? Critics point out that allocating farmland for this use might drive up the price of food crops.

Limestone

Marine organisms take dissolved carbon out of the water and use some of it to make their carbonate shells. As we saw earlier in this chapter, the carbon can be in the form of carbon dioxide dissolved in the water or it can be in the form of hydrogen carbonate ions. The organisms that build coral reefs are called coral polyps, and they absorb two ions from the seawater to build the reef: hydrogen carbonate ions and calcium ions. When combined, molecules of calcium carbonate ($CaCO_3$) are formed. This molecule is the basis of the coral reef, and it is sturdy like rock. Below is the chemical equation for making calcium carbonate:

$$Ca^{2+} + 2\,HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

Other organisms as well as coral polyps use calcium carbonate to build shells around their bodies. Molluscs (from the phylum Mollusca), such as snails, clams, oysters, and mussels, build up their shells with calcium carbonate and, when they die, the shells accumulate at the bottom of the ocean.

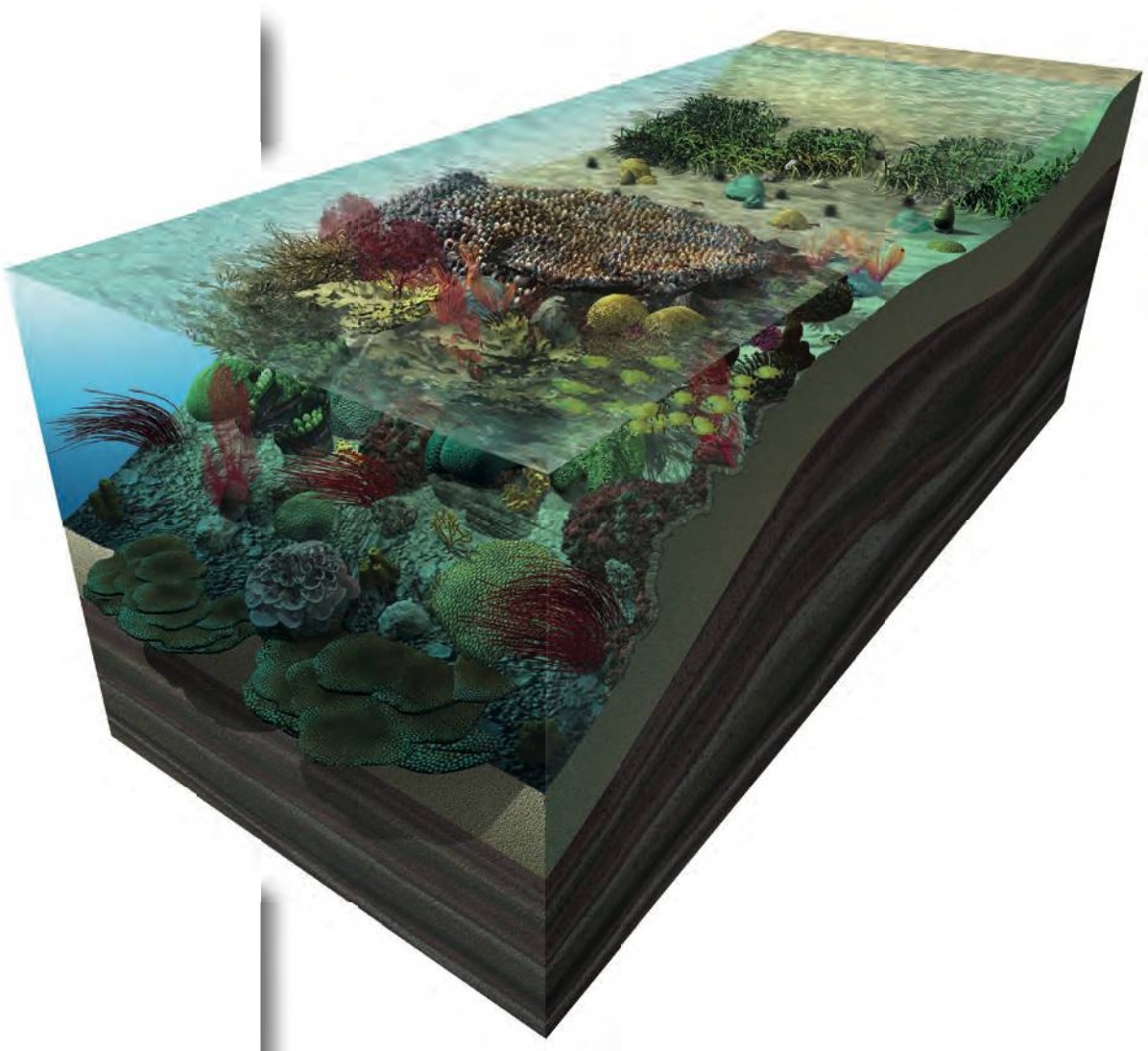


Figure 4.15 Coral reefs are formed from dissolved calcium and carbonate ions found in ocean water.

Microscopic foraminifera usually live on the ocean floor and are also very good at building shells, albeit very small ones. Because they are so numerous, however, and they have been around for hundreds of millions of years, their shells have accumulated in sediments, and when the sediments go through the process of lithification, they form limestone. Limestone has long been used by humans as a building material (the Great Pyramid at Giza and Notre Dame cathedral in Paris are two examples), and is a major ingredient in modern cement.

The process of taking carbon out of the environment and 'locking it up' in a substance for an extended period of time is called carbon sequestration, and when it happens naturally it is called biosequestration. This is one way balance is maintained in the carbon cycle.

Through biosequestration, an accumulation of foraminifera shells as sediments at the bottom of the ocean can trap carbon in limestone for millions of years. When cement is made by humans for construction, limestone is used and, in the process, some of the carbon is released back into the atmosphere as carbon dioxide, cancelling out the biosequestration.

CHALLENGE YOURSELF

4 Using Table 4.4, draw a flowchart showing the exchange of carbon between the atmosphere, the oceans, and the biosphere. Such exchanges are called fluxes and carbon fluxes in this table are expressed in gigatonnes of carbon per year (GtC yr^{-1}). You can do the drawing by hand but there are many flowchart tools available, both online and probably as part of the software on the computer you use.

Carbon fluxes	Quantity of carbon (GtC yr^{-1})
Examples of fluxes into the atmosphere	
Respiration of terrestrial organisms	120
Respiration of marine organisms at the surface of the ocean	92
Burning of fossil fuels (such as transport)	7.7
Changes in land use (such as deforestation)	1.5
Examples of fluxes out of the atmosphere	
Absorption of carbon dioxide into the water at the surface of the ocean	90
Gross primary production (GPP), photosynthesis of terrestrial organisms	90
Photosynthesis of marine organisms	40
Changes in land use (such as growing crops in prairies)	0.5
Weathering, carbon dioxide being incorporated into rocks and soils	0.2

Table 4.4 Carbon exchange into and out of the atmosphere

Exercises

7 Study Figure 4.16.

- **(a)** Using the dark blue trend line, determine the atmospheric carbon dioxide concentration for 1965 and 2001.
- **(b)** Calculate the percentage change from 1965 to 2001.
- **(c)** Why do the measurements have a high point and a low point for each year?
- **(d)** The photo insert shows the station where the measurements were taken, at the top of a volcanic island in the Pacific that is part of a USA state. Which state is the station in, and why did scientists decide to put the station there?

8 From what inorganic molecules can aquatic organisms get their carbon?

9 Give the names of the hydrocarbon-rich substances that are described below.

- **(a)** A kind of waterlogged soil found in wetlands and made of partially decomposed plant material.
- **(b)** A hard black rock that can be burned to make electricity or direct heat.
- **(c)** A waxy substance formed from accumulated lipids trapped in sediments at the bottom of oceans.
- **(d)** Of all the commonly used petroleum products, this one has the smallest density.

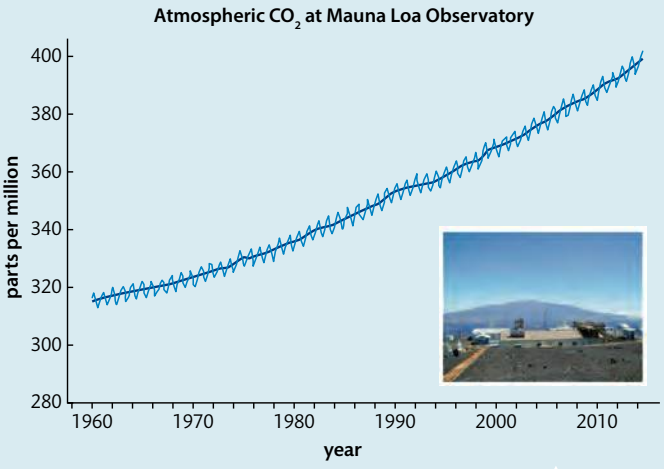


Figure 4.16 The National Aeronautics and Space Administration (NASA) data on carbon dioxide levels in the atmosphere 1958–2014. The up and down pattern is caused by seasonal fluctuations in activities such as photosynthesis. Dr Pieter Tans, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/) and Dr Ralph Keeling, Scripps Institution of Oceanography (scrippsco2.ucsd.edu/)

Worked example

You be the scientist: have a look at this graph from the website of the National Oceanic and Atmospheric Administration (NOAA), showing atmospheric carbon dioxide levels in recent years. This one is from October 2013, but you might be able to find a more recent one if you do a web search for the title.

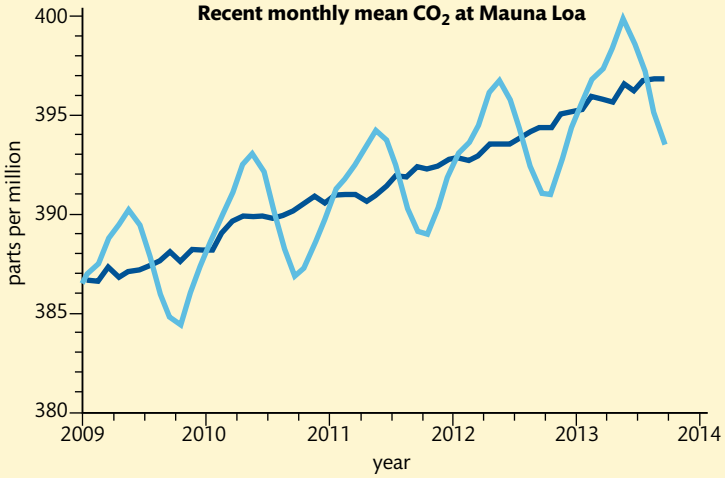


Figure 4.17 Levels of atmospheric CO_2 from 2009 to 2014. Dr Pieter Tans, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/) and Dr Ralph Keeling, Scripps Institution of Oceanography (scrippsco2.ucsd.edu/)

The up-and-down pattern shown in light blue is caused by seasonal fluctuations in carbon dioxide levels. The dark blue line shows the trend corrected for these seasonal fluctuations.

1 **(a)** Work out how the years are divided up on the *x*-axis of the graph.

(b) Estimate the level of atmospheric carbon dioxide in January of 2010 and in October of 2013 using the corrected values on the dark blue trend line.

(c) Look at the first four lowest values on the light blue line. There is one per year. Determine the month of the year during which this low point most often occurs. Do the same for the high points.

2 **(a)** In terms of cellular respiration and photosynthesis rates in the northern hemisphere, explain the yearly downward fluctuations from May to October.

(b) Do the same for the upward fluctuations from October to May of the following year.

3 Describe the overall trend shown by the graph for the years shown, giving quantitative data in your description.

Solutions

1 **(a)** The years are divided into quarters: Jan/Feb/Mar, Apr/May/Jun, Jul/Aug/Sep and Oct/Nov/Dec.

(b) 388 p.p.m. and 397 p.p.m., respectively. It is important to include the units.

(c) Lows are in October and highs in May.

2 **(a)** As plants, phytoplankton, and photosynthetic bacteria are generally more active in the spring and summer months, more carbon dioxide is extracted from the atmosphere and levels drop. During this time, cellular respiration is contributing large quantities of carbon dioxide to the atmosphere, but not as fast as photosynthesis is taking it out.

(b) Conversely, when photosynthesis is less intense during the autumn and winter months, carbon dioxide levels rise and, although organisms are generally less active at colder times of the year, their cellular respiration rates put more carbon dioxide into the air than the photosynthetic organisms can remove.

3 The trend shows an increase from 387 p.p.m. at the beginning of 2009 to a 397 p.p.m. in October 2013. This 10 p.p.m. increase represents a percentage change of +2.6% for the period shown.

4.4 Climate change

Understandings:

- Carbon dioxide and water vapour are the most significant greenhouse gases.
- Other gases including methane and nitrogen oxides have less impact.
- The impact of a gas depends on its ability to absorb long-wave radiation as well as on its concentration in the atmosphere.
- The warmed Earth emits longer wavelength radiation (heat).
- Longer wave radiation is absorbed by greenhouse gases, which retain the heat in the atmosphere.
- Global temperatures and climate patterns are influenced by concentrations of greenhouse gases.
- There is a correlation between rising atmospheric concentrations of carbon dioxide since the start of the industrial revolution 200 years ago and average global temperatures.
- Recent increases in atmospheric carbon dioxide are largely due to increases in the combustion of fossilized organic matter.

Applications and skills:

- Application: Threats to coral reefs from increasing concentrations of dissolved carbon dioxide.
- Application: Correlations between global temperatures and carbon dioxide concentrations on Earth.
- Application: Evaluating claims that human activities are not causing climate change.

Guidance

- *Carbon dioxide, methane, and water vapour should be included in discussions.*
- *The harmful consequences of ozone depletion do no need to be discussed and it should be made clear that ozone depletion is not the cause of the enhanced greenhouse effect.*

NATURE OF SCIENCE

Assessing claims: assessments of the claims that human activities are not producing climate change.

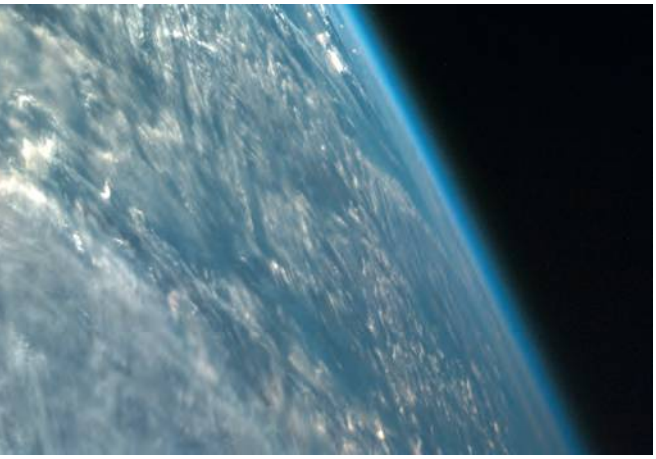
The atmosphere

We live at the bottom of an ocean of air we call the atmosphere. It is so natural to us that we don't even think about it unless, for some reason, we are without it. When we go up in an airplane, for example, the cabin needs to be pressurized so that we can keep breathing, and so that we do not freeze to death 10 000 m above the ground. The atmosphere plays a vital role in regulating the temperature of Earth's surface.

Earth's surface has an average temperature of about 14°C; fluctuations only very rarely go lower than –80°C (in Antarctica) or higher than +50°C (in North Africa). In contrast, the Moon, which is the same distance from the Sun as Earth is, has temperature swings that typically go from –150°C to +120°C, depending on where sunlight is hitting the surface. This is because the Moon has almost no atmosphere. It is estimated that if Earth had no atmosphere, the average temperature would be 32°C colder (–18°C), making the possibility of life very different. We will see in this section how Earth's atmosphere acts as a kind of blanket, keeping us warm at night and sheltering us from excessive heat during the day.

The roles of carbon dioxide and water vapour in the greenhouse effect

The consequence of the Moon having little or no atmosphere is that it has no greenhouse effect. The greenhouse effect refers to a planet's ability to use its atmosphere to retain heat and keep warm even when no sunlight is hitting the surface. To understand the greenhouse effect, you need to know how a greenhouse works. The walls and roof of a greenhouse are made of glass. Sunlight penetrates through the



Seen from space along the edge of Earth's curve, the atmosphere is a surprisingly thin, almost insignificant looking, layer of gases.

glass and warms up the plants inside. Sunlight itself, which is made up of short wavelengths, is not warm; the temperature of outer space between the Sun and Earth is hundreds of degrees below freezing.

It is only when sunlight hits an object that some of its energy is transformed into heat. Heat energy, otherwise known as infrared radiation, has longer wavelengths than energy in the form of light. When sunlight goes through the glass of the greenhouse, it warms up the objects inside: the plants, the ground, and anything else inside. The objects inside radiate their heat to the air inside the greenhouse, but the glass of the greenhouse is not as transparent to heat energy as it is to light energy, so some of the heat is then trapped inside the greenhouse. The glass also plays a major role in preventing warm air from rising through convection to dissipate the heat. The result is that the temperature inside the greenhouse is warmer than outside. This helps plants to grow better when it is cold outside, which is one of the main reasons why farmers and gardeners use greenhouses.

Even if you have never been inside a greenhouse, you have probably felt the greenhouse effect when getting into a car that has been sitting in the sunshine with its windows closed on a hot day. The greenhouse effect on a planet is not caused by glass windows, but by its atmosphere's ability to retain heat in a similar way to that of the glass of a greenhouse or car.

The inside of a greenhouse

Greenhouse gases (GHGs), such as water vapour and carbon dioxide in Earth's atmosphere, can be thought of as the glass of a greenhouse, although, like many models, this is not a very accurate representation of the natural phenomenon. GHGs have the ability to absorb and radiate infrared radiation (heat). When such gases are present, they keep the atmosphere near Earth's surface warm by absorbing heat from the warmed surface and re-radiating it in all directions, including back down towards the surface. In addition to carbon dioxide and water vapour, methane and nitrogen oxides also contribute to Earth's greenhouse effect, but to a lesser extent.

Climate experts at the International Panel on Climate Change (IPCC) have confirmed that Earth is undergoing global warming because of an enhanced greenhouse effect, also known as the runaway greenhouse effect. Increasing levels of some of the main greenhouse gases (as a result of human activities, such as burning fossil fuels) are causing the atmosphere to retain more and more heat. There will be more about how this works later.

Different gases, different impacts

Different gases in the atmosphere have different impacts on the greenhouse effect on Earth. There are two main factors that determine how much of an influence a gas will have on the greenhouse effect:

- the ability of the gas to absorb long-wave radiation (heat)
- the concentration of that gas in the atmosphere.

Methane, for example, actually has a much greater potential to warm the planet than carbon dioxide, but methane has a relatively short lifetime in the atmosphere: approximately 12 years. Carbon dioxide has an estimated lifetime of 50–200 years in the atmosphere. This is because methane can be broken down into other molecules, whereas carbon dioxide is not very reactive and so can stay in the atmosphere for much longer.

Studies of increases in carbon dioxide and methane gases over time have revealed that carbon dioxide concentrations have increased by approximately 40% since 1750, while methane concentrations have increased by more than 150% in the same time period. However, methane concentrations in Earth's atmosphere are about 1700 p.p.b. (parts per billion) whereas carbon dioxide concentrations are about 400 p.p.m. (parts per million), meaning that the concentration of carbon dioxide is more than 200 times greater than that of methane.

This huge difference is the main reason why environmental groups and government policy makers are much more interested in carbon dioxide concentrations than methane concentrations, although both need to be taken into account in discussions about global climate change. Nitrogen oxides represent just over 320 p.p.b., so they are about a fifth the concentration of methane and, even though they have a global warming potential more than 100 times that of carbon dioxide, their concentration in the atmosphere is more than 1000 times smaller than carbon dioxide concentrations, so they are less of a concern.

The warmed Earth emits longer wavelength radiation (heat)

As we have seen with a greenhouse, when sunlight touches an object inside, some of the light energy is absorbed and converted into heat energy, also known as long-wave infrared radiation. On Earth, the mountains, forests, rivers, and oceans absorb some of the sunlight and are warmed. Most of the sunlight bounces off the surface and goes back into space. This is what makes photos such as the one at the top of page 204 possible. Only a small amount is converted into infrared to warm up the surface.

The ability of a surface to reflect light is called its albedo. Light-coloured objects, such as ice and white sand, have a high albedo, so very little light is absorbed and such objects do not heat up as much as dark objects such as dark-coloured rocks and black sand. Think about walking barefoot on light-coloured cement on a hot and sunny day, compared with walking barefoot on black asphalt on the same day. Dark-coloured substances such as the asphalt have a low albedo, and absorb lots of light and convert it into heat.

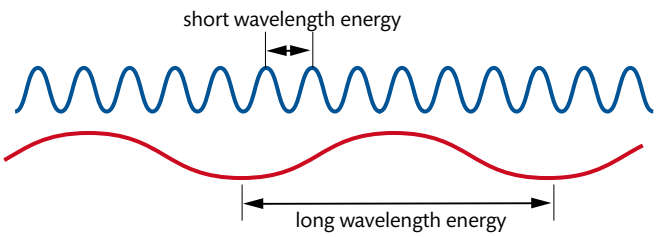


Figure 4.18 Different wavelengths of energy have different properties.

Governments all over the world have been looking at various possibilities for preventing climate change from getting worse. Over the years, efforts such as the Rio Summits in 1992 and 2012, and the Kyoto Protocol in 1997, have tried to establish goals for carbon emissions. More recently, ideas of a 'carbon tax' or 'cap and trade' policies have been put forward, so that countries compensate for their excessive carbon emissions. Fast-growing economies such as China and India have been under scrutiny for their exponential increases in energy needs, and have been criticized by industrialized nations for using non-renewable energy sources such as coal, which produce excessive carbon dioxide emissions. It can be considered curious that industrialized countries that for centuries have built their economies on carbon dioxide-emitting fossil fuels would tell countries that are more recently following such economic development that they cannot do the same. It will be interesting to see whether countries all over the world will continue to burn fossil fuels until the last lump of coal or the last drop of crude oil is gone. Then again, perhaps international agreements will curb fossil fuel use and prevent climate change from getting worse.

Figure 4.19 A summary of the greenhouse effect: short-wave radiation (shown in yellow) hits the surface and some is converted into long-wave radiation (shown in orange). Some of this infrared heat escapes into space but some (shown in red) is radiated back by greenhouse gases.

How greenhouse gases heat the atmosphere

If Earth had no atmosphere, the heat radiating from low albedo objects on its surface would simply radiate back into space, and at night we would see temperatures plunge to ones similar to the extremely cold temperatures on the Moon.

The reason that this does not happen is because the greenhouse gases absorb and retain the infrared radiation coming from the surface. The greenhouse gases can then re-radiate the heat in all directions, the way a radiator does in a cold room. Some of this heat will be lost to space, but some of the long-wave radiation will be directed down to the surface, keeping it warm. The rest will radiate within the atmosphere, preventing it from getting extremely cold at night when no more sunlight is present. When the Sun rises again in the morning, the surface will heat up and the whole process starts again.

During the winter season, the days are shorter and the angle of sunlight is less direct, so Earth's surface cannot warm up as much. This is why it is colder in the winter. In the summer, days are longer and the sunlight hits Earth's surface more directly and intensely. Earth's surface can get very hot, and, during heat waves, the nights are not cool enough to cause the daytime temperatures to lower.

Fortunately, certain gases in the atmosphere filter out some of the more harmful radiation from the Sun, such as UV radiation. Because the atmosphere filters the sunlight, not all of it reaches the surface. This prevents the surface from getting as hot as the Moon's maximum temperature of +120°C, even on the hottest of summer days. So you can see how the atmosphere acts as a kind of blanket around the planet: at night it keeps the planet warm, and during the day it provides a barrier protecting life from too much solar radiation.

Global climate change is affected by greenhouse gases

Climate refers to the patterns of temperature and precipitation, such as rainfall, that occur over long periods of time. Whereas weather can change from hour to hour, climates usually do not change within a human's lifetime: climate changes generally occur over thousands or millions of years. Climatologists and palaeoclimatologists collect data about atmospheric conditions in recent decades and the distant past, respectively. As thermometers have only been around for a few hundred years, temperatures on Earth from thousands or millions of years ago must be inferred from proxies. (See Nature of Science box on the next page for more about proxies.)

Proxy data show that, in the northern hemisphere 15 000 years ago, it was very cold, and Earth was undergoing a glaciation, or ice age. Ice ages were periods of significant change in climate that produced sheets of ice hundreds of metres thick in regions where today there are thriving cities. For example, in the geographical location that is now Berlin in Germany, there would have been an ice sheet similar to the ones still sitting on Greenland and Antarctica today. The last ice age ended about 10 000 years ago, and we are now in an interglacial period associated with warmer temperatures. It does not take much of a temperature drop to produce a glaciation: it is estimated that the last ice age was caused by a global average temperature reduction of 5°C. By looking at deeper ice cores, we know that there has been a succession of ice ages over millions of years.

Because the greenhouse effect is often misunderstood, be sure to master the scientific vocabulary and concepts. This is challenging for students and adults alike. Few people can explain it precisely, too often saying something incorrect such as 'sunlight is trapped in the air'.

NATURE OF SCIENCE

How can scientists determine a quantitative value for something that is not directly measurable? The answer is by using a proxy, which is a measurement that is used in place of another one. Because it is impossible to go back in time and measure the temperature of the atmosphere 15 000 years ago, climatologists use proxies, such as tree rings, coral reef growth, and the presence of fossils of temperature-sensitive organisms, to estimate the climate back then. By digging in layers of sediment 15 000 years old and looking at the kinds of bones, shells, coral reefs, plant fossils, and even pollen grains, climatologists can work out what the climate was like at that time in the past. Certain species of foraminifera microfossils, for example, can reveal temperature changes via slight changes in the chemical compositions of their shells.

An ice core being removed from the drilling apparatus.

Layers found in thick sheets of ice that have been formed by annual snowfall can also be used in a similar way as tree rings and ocean sediments. By drilling into the ice and taking cylinder-shaped samples, called ice cores, scientists can study the substances trapped in the layers, such as air bubbles from the year when the layer was deposited. Researchers at Vostok Station in Antarctica have collected layers of ice from more than 3000 m down, yielding climate information going back more than 400 000 years. One indication of temperature is the frequency of different types of isotopes (versions of atoms) found in the air bubbles. Oxygen atoms, for example, are usually found in their most abundant isotope, which is oxygen-16, but can also be found in their 'heavier' form, oxygen-18, which has a greater mass because it has two extra neutrons. When glaciations happen, the oceans have a slightly higher ratio of oxygen-18, and the glaciers that form have a slightly higher ratio of oxygen-16. By examining these ratios in ice and in the shells of marine fossils, climatologists can trace the colder and warmer periods of the past.

CHALLENGE YOURSELF

5 The graph below shows the results of collecting data representing thousands of years trapped in ice core samples.

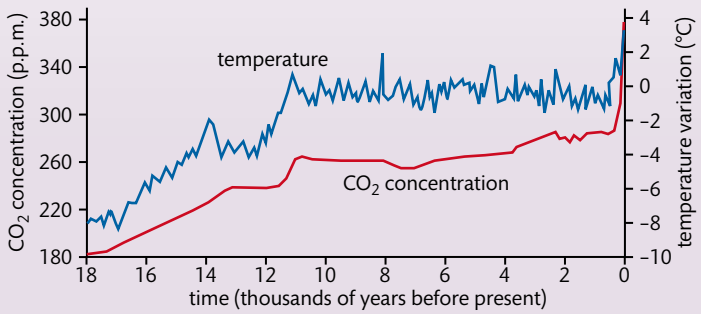


Figure 4.20 Ice core data.

The red line on the graph shows carbon dioxide concentrations that were measured from air bubbles trapped in the ice.

The blue line shows fluctuations between warmer temperatures that are close to zero (representing no change from modern climatic conditions) and colder temperatures several degrees below what they are today.

(a) Is there a strong or a weak correlation between carbon dioxide levels and atmospheric temperatures over the last 400 000 years?

(b) Can scientists conclude that there is causality from this graph: that rising carbon dioxide levels cause global temperatures to go up?

(c) What further evidence would be necessary to confirm or refute causality?

Earth has shown many fluctuations in global temperatures over millions of years. Such fluctuations happened long before humans started producing excessive greenhouse gases. The changes being observed now are alarming scientists because they are happening so quickly and cannot be explained by natural phenomena.

Many factors are thought to contribute to global temperature changes over time, for example volcanic activity and particles suspended in the air, the quantity of radiation from the Sun, the position of the continents (which move on plates over millions of years), oscillations in ocean currents, fluctuations in Earth's orbit and the inclination of its axis, and probably other phenomena that are yet to be discovered. However, in this chapter we are only going to focus on the influence of changes in the composition of the atmosphere, notably the presence of greenhouse gases.

As shown in Figure 4.20, there appears to be a strong correlation between temperature increase and carbon dioxide increase. Knowing the properties of greenhouse gases, as discussed earlier, it is clear that an increase in carbon dioxide levels will lead to warming of the atmosphere, because it would increase the greenhouse effect. Having said this, closer inspection of the data shows that the increase in temperature (in blue) happens first and then the carbon dioxide concentration (in red) rises. This lag time is partly explained by the fact that, as oceans warm up, they release carbon dioxide, because gases dissolve less well in warm water than in cold water. A positive feedback loop leads to further increases in temperatures over time: warmer temperatures → more carbon dioxide → even warmer temperatures → even more carbon dioxide, and so on.

NATURE OF SCIENCE

Want to see the data for yourself? One of the principles of science, especially research funded by taxpayers, is to make data available to the public. This is to allow verification, critique, and sharing of data, so that scientists with many different approaches can combine their findings and advance our understanding of the topics being studied.

One organization that does this is NOAA. If you go to the NOAA Earth System Research Laboratory Global Monitoring Division's website (see the hotlinks at the end of this section), you will find maps, graphs, and databases of measurements of carbon dioxide and other atmospheric gases over many decades. Check out the section called Products.

How do scientists know that the current situation is exceptional, that the changes in Earth's atmosphere are being caused by human activities and are not just part of a natural phenomenon?

TOK

The industrial revolution

Ever since machines started replacing hand tools in Europe in the 1800s, humans have produced increasing quantities of carbon dioxide from factories, transport, and other processes using fossil fuels, notably coal and oil. In addition, burning forests to make way for farmland and burning wood for cooking and heating has contributed to this increase.

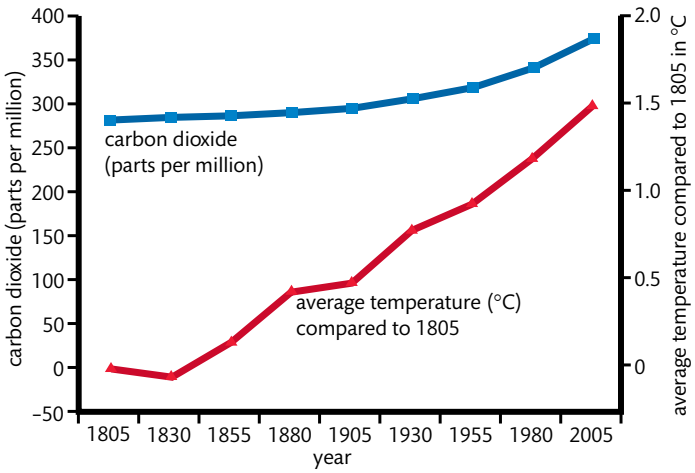


Figure 4.21 Two hundred years of atmospheric changes.

Over the decades, human activities have produced enough carbon dioxide to considerably raise the percentage of this gas in the planet's atmosphere. Estimates suggest that the level of carbon dioxide in the atmosphere has increased by more than 35% compared with its pre-industrial revolution levels.

Recent increases in atmospheric carbon dioxide are largely due to increases in the combustion of fossilized organic matter

The gases produced by human activity that retain the most heat are among the ones we have already identified as greenhouse gases: carbon dioxide, methane, and oxides of nitrogen. The concentrations of these gases in the atmosphere are naturally low, which normally prevents too much heat retention.

The number one source of carbon emissions as a result of human activity is transport that is based on fossil fuels: cars, lorries, diesel trains and airplanes. Other human activities that put carbon dioxide into the air include the following: deforestation, heating homes by burning fossil fuels, maintaining a diet high in meat (the meat industry is highly dependent on fossil fuels), purchasing goods that have to be transported long distances from where they are produced to where they will be used, travelling long distances between work and home, purchasing foods that are grown out of season in greenhouses heated by fossil fuels.

Each vehicle produces its own mass in carbon dioxide every year.

Human activities contribute to the production of other greenhouse gases. Again, diet has an impact here, this time with the production of methane. Remember that methane is produced by anaerobic microorganisms present in the guts of animals. Mass consumption of meat, especially in the USA, where people eat the most meat per person per year, has led to an increase in the number of cattle being raised. Cattle are responsible for producing large amounts of methane that escape into the atmosphere.

Lastly, oxides of nitrogen (NO_x) are produced by human activities such as:

- burning fossil fuels (e.g. gasoline in cars) and using catalytic converters in exhaust systems
- using organic and commercial fertilizers to help crops grow better
- industrial processes (e.g. the production of nitric acid).

Consumer demands push industries to produce more, which means burning more energy and releasing increasing amounts of greenhouse gases.

Consumer demands for wood products such as housing, firewood, furniture and paper lead to massive deforestation.

Do not confuse the greenhouse effect with the depletion of ozone. Although both are the result of human activity, and both influence the atmosphere, they are not interchangeable phenomena. They have different causes and different effects on the environment.

The problem is that human production of greenhouse gases shows little sign of slowing. As consumer demands for fuel and food increase, so the excess production of waste gases increases.

NATURE OF SCIENCE

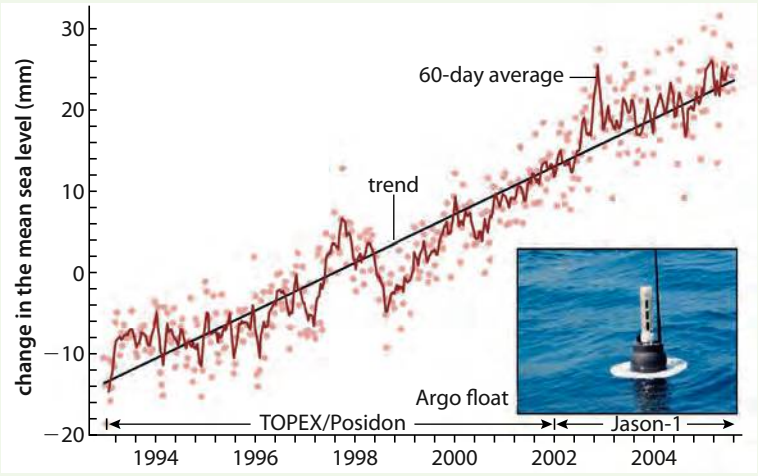


Figure 4.22 NASA data on sea level and the Argo marker, which is used to make the measurements.

How do scientists collect data to see climate change? Look at the various types of data on this page. What kinds of technology are necessary in order to collect the data? How do these data contribute to our understanding of climate change? Large portions of this chapter have dealt with carbon dioxide concentrations: how and where are they collected? Do stations in different parts of the world agree or disagree about trends in carbon dioxide emissions?

This is a satellite photo of forest fires in the Yucatan peninsula in 1998. The forests were drier than usual that year. Forest fires release large quantities of carbon dioxide into the atmosphere.

Threats to coral reefs

The organisms that build coral reefs are very sensitive to the following: water temperature, water acidity, and the depth of the water. Unfortunately, all three factors are changing in the oceans of the world as a result of human activities. Increased carbon dioxide concentrations in the air lead to increased dissolved carbon dioxide in the oceans, which lowers the pH of seawater. When it is intense, ocean acidification leads to the death of coral polyps and algae, and when they die the reefs are not built up anymore. As a result, the colour of the reef goes from being richly multi-coloured to being as white as bone. This coral reef death is called bleaching and it interrupts the food chain, causing many of the organisms that live there to seek food and shelter elsewhere. Similar to a forest that has lost all its leaves because of acid rain, a bleached coral reef can no longer support the rich ecosystem that once lived there.

Are humans causing climate change?

Not everyone is convinced that climate change is happening, or that it is caused by human activity. Such critics are sometimes referred to as 'climate change deniers', and they have a number of criticisms about the IPCC's findings.

How do scientists respond to such criticism? What arguments and justifications do they use in response? Table 4.5 presents a few.

Table 4.5 Opinions of climatologists and their critics

Challenges from critics	Possible responses from climatologists
Climate change as a result of human activity is just a theory, not a fact.	Evidence clearly shows temperature increases since the industrial revolution. Decades-old predictions of extreme weather events, record temperatures, and receding glaciers are being confirmed day after day. Climate change is not a debate or a controversy: it is well-supported by an increasing volume of data. The findings of the IPCC state, 'The largest contribution to total radiative forcing is caused by the increase in the atmospheric concentration of CO_2 since 1750.' The term 'radiative forcing' means the difference between the energy arriving at the surface and the energy being lost into space. And the increase in CO_2 referred to is clearly traced to human activity.
There is disagreement within the scientific community about human-induced climate change. Many scientists disagree and have published research showing that climate change is not due to human activity.	The vast majority of recent publications from climatologists confirm anthropogenic climate change; there is a consensus in the scientific community. Often the dissenting scientists who are quoted by critics are not climatologists, or they are quoting out-of-date or refuted data. In other instances, scientists only disagree on the quantity of change or on the amount of responsibility of human activity.

Challenges from critics	Possible responses from climatologists
Your models predicted even more of a temperature increase than is actually happening. How can you explain that? Human activities such as burning fossil fuels are increasing, so why aren't the temperatures increasing equally fast?	Like any science, climatology is complex and we are learning new things all the time. For example, human activities such as transport produce particles in the air that remain in suspension, and some of these aerosols can diffuse sunlight, causing a reduction in the amount of short-wave solar radiation reaching the surface. Less solar radiation hitting Earth means lower temperatures because there are fewer rays of short-wave radiation reaching the surface to be converted into infrared. This phenomenon is cancelling out some of the predicted warming.
There have been huge fluctuations in climate in the past, and the current changes that we are seeing in recent decades are natural. For example, the Sun is currently in a phase of high-energy output. Wouldn't that be a more logical explanation?	Although it is true that Earth's climate has seen warming and cooling in the past, those changes were relatively slow, taking place over thousands or millions of years. The changes that we are seeing now are happening on a scale of decades, and the speed and magnitude at which CO_2 levels and temperature are increasing are unprecedented. One concrete example of the consequences are so-called 100-year storms that, instead of happening once every century, are occurring several times within the same decade. As for the Sun's output: yes, it is currently in a high-output phase, but that extra energy has only a small fraction of the effect that human-induced global warming has. Also, the most recent hottest years on record happened during a period of lower solar output.
Insisting that climate change is caused by human activity means that, to solve the problem, we are going to need to reduce CO_2 emissions. That will have a severe negative economic effect, as many carbon-based industries will lose revenue.	The alternative, if we let people introduce more and more greenhouse gases into the atmosphere, will exacerbate the already highly destructive patterns we are seeing, and the cost of fixing these new problems is difficult to imagine. Enormous economic burdens are presented by problems such as the damage caused by an increase in extreme weather events, such as super storms and hurricanes, rising sea levels, droughts as well as flooding, a reduction of snow at high altitudes influencing melt water supplies downstream, to name a few.

In the end, climatologists make a clear distinction between what is politically controversial and what is scientifically controversial. The political debate is often driven by non-scientific arguments. One way to spot non-scientific arguments is to look for whether or not the proponent's comments are motivated by economic arguments, notably when they are motivated by their affiliation with industries that produce large quantities of carbon dioxide. As such industries would potentially lose revenue if limits were put on carbon dioxide emissions, it is in their interest to promote doubt and controversy.

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Climate change raises many issues about how science works. Here are four to consider.

1. The fact that there are sceptics and critics of the IPCC reports on global climate change is a good thing. Science encourages constructive criticism and verification, and is open to modification if the criticisms are valid. Often errors and misinterpretations of data are spotted when many people read a publication, and this pushes scientists to be more precise and to be better communicators.
2. The IPCC report is filled with qualifying statements such as 'likely', 'highly likely', 'extremely likely' about the future. Why can't IPCC just make up its mind and say that something is sure to happen? Because systems such as global climate are complex, scientists do not fully understand how they work and, although they are regularly gaining further insights, sometimes they are wrong. For example, the predictions of how fast global temperatures will increase seem to have been confirmed for some years but not for others.
3. Climate change deniers will grab onto such inaccurate predictions and say, 'See? Your models are wrong. Therefore, no one should listen to you.' This is an example of cherry picking, something both sides of the debate are accused of doing. Cherry picking is a form of confirmation bias that consists of only looking at the evidence supporting your side of the argument, and ignoring or downplaying the evidence that hurts your argument. Both sides of the debate have been accused of following blind faith rather than objectively assessing the evidence.
4. Scientists need money for their work, and they often get that money from grants offered by governments and industries. If a scientist is getting funding from an organization that promotes the preservation of nature, the chances are reasonably good that that scientist will tend to look for evidence of human-induced climate change, whereas a scientist whose funding comes from industries highly reliant on fossil fuels will probably tend to look for evidence against human-induced climate change. Journalists and citizens need to be vigilant about this, and double-check where the interpretations of the data are coming from.

CHALLENGE YOURSELF

6 Do you know your carbon footprint? This is the amount of carbon dioxide you as an individual are contributing to the atmosphere. There are many online 'Footprint calculators' available, notably from the Nature Conservancy and WWF. Use the hotlinks at the end of this section to try one: what do you get, and how do you compare with the rest of the world? In what ways are you willing to try to reduce your carbon footprint: diet, transport, home energy use? The website that calculates commuters' itineraries on the Metro system in Paris, France, also calculates how much carbon is saved by not burning fossil fuels for the same commute. Does your public transport system's website have a similar calculator?

Too often people think that climate change is someone else's doing, that their personal day-to-day decisions do not have an impact.

TOK

When playing a board game with family or friends, cheating is frowned upon. If one player took more turns or more points than the rules allowed, that person would be considered a cheater and might be asked to leave the table. If everyone around the table started cheating, the game would break down completely. Are there similar situations in society? For example, if a few people break the law, they are often treated as criminals and punished; but if everyone cheated all the time, society would break down. Are there any parallels with pollution? If people are knowingly polluting and not doing anything to reduce their carbon footprint, are they treated by society as cheaters? There are enough warning signs to lead experts to invoke the precautionary principle. This is an ethical theory that says that action should be taken to prevent harm even if there is not sufficient data to prove that the activity will have severe negative consequences. It also stipulates that if people wish to engage in an activity that may cause changes in the environment, they must first prove that it will not do harm.

Without the precautionary principle, industries and consumers tend to proceed with their activities until it becomes clear that harm is being done to the environment. When irrefutable proof is provided, usually action is taken to reduce the activity in question. For example, the use of the pesticide DDT was prohibited in North America when it was proven to accumulate in ecosystems and reduce populations of birds of prey such as the bald eagle. That decision saved the bald eagle from extinction. How can we 'prove' that something is safe for the environment?

With regards to global warming, tenets of the precautionary principle say that preventative action should be taken now to reduce carbon emissions and greenhouse gas production before it is too late. In addition, the principle holds that those who wish to continue producing excess greenhouse gases should prove that there are no harmful effects before continuing.

In response, farmers, manufacturers, and transport providers, among others, wonder why they should invest money in new techniques that reduce greenhouse gases if scientists are not 100% sure how an enhanced greenhouse effect is going to be harmful to the environment. Industries that make the effort to invest in such measures may find themselves less economically viable than their polluting competitors.

Consequently, unless preventative measures are taken across the board by countries worldwide, there will always be polluting competitors who can offer products at a lower price. The risk is that they will drive the ecologically conscious companies out of business because they do not use any of their capital on ecological measures.

Ideally, well-informed consumers could choose products or services that are provided by ecologically minded companies. If this is done on a massive scale, companies would provide eco-friendly products and services to attract customers, and those companies that did not would be shunned as rogue companies by consumers and be driven out of business.

To learn more about NOAA and carbon footprints, go to the hotlinks site, search for the title or ISBN, and click on Chapter 4: Section 4.4.

Exercises

10 Distinguish between how a garden greenhouse works and how the greenhouse effect on Earth works.

11 Of the greenhouse gases discussed in this chapter, state which one has a warming potential approximately 100 times that of carbon dioxide. Why aren't scientists talking more about this if it has such a potential to increase the greenhouse effect?

12 In what ways could you reduce your consumption of fossil fuels on a day-to-day basis?

13 A scuba diver returns to her favourite coral reef only to find it empty of life and all the corals turned white. She asks you if you know what this phenomenon is: what do you tell her?

Practice questions

1 What is a community?

A A group of producers and consumers living and interacting in an area.

B A group of species living and interacting in an area.

C A group of organisms living and interacting in an area.

D A group of populations living and interacting in an area. *(Total 1 mark)*

2 The scarlet cup fungus, *Sarcoscypha coccinea*, obtains its nutrition from decaying wood by releasing digestive enzymes into the wood and absorbing the digested products. Which of the following terms describe(s) the fungus?

I. Autotroph

II. Heterotroph

III. Saprotroph

A III only.

B II and III only.

C I and III only.

D I, II, and III. *(Total 1 mark)*

3 Why do food chains in an ecosystem rarely contain more than five organisms?

A Nutrients are recycled by the decomposers back to the producers.

B Nutrients are lost from the ecosystem when organisms die.

C The conversion of food into growth by an organism is not very efficient.

D Energy is recycled by the decomposers back to the producers. *(Total 1 mark)*

4 Several greenhouse gases occur in the atmosphere. Carbon dioxide (CO_2) is one of them but so are methane (CH_4) and oxides of nitrogen (NO_x). Why are oxides of nitrogen classed as greenhouse gases?

A They trap some of the long-wave radiation emitted by Earth's surface.

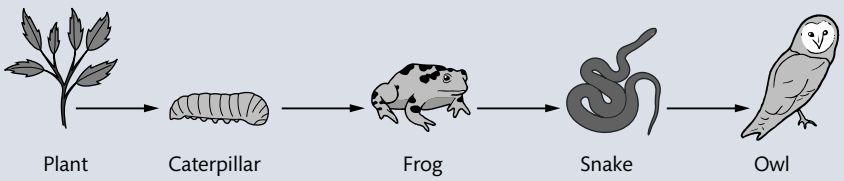
B They prevent short-wave radiation from reaching Earth's surface.

C They dissolve in rainwater to produce acid rain.

D They are only produced by human activity whereas CO_2 and CH_4 are also produced naturally. *(Total 1 mark)*

5 Explain the shape of the pyramids of energy that are constructed by ecologists to represent energy flow in an ecosystem. *(Total 3 marks)*

6 This diagram represents a simple food chain. In which ways is energy lost between the trophic levels?



I. Heat loss through cell respiration.

II. Material not consumed.

III. Material not assimilated.

A I and II only.

B I and III only.

C II and III only.

D I, II, and III. *(Total 1 mark)*

7 Describe the relationship between the rise in the concentration of atmospheric carbon dioxide and the enhanced greenhouse effect. *(Total 5 marks)*

8 Outline the precautionary principle. *(Total 2 marks)*

05 Evolution and biodiversity

Essential ideas

5.1	There is overwhelming evidence for the evolution of life on Earth.
5.2	The diversity of life has evolved and continues to evolve by natural selection.
5.3	Species are named and classified using an internationally agreed system.
5.4	The ancestry of groups of species can be deduced by comparing their base or amino acid sequences.

Lemurs arrived on the Comoro Islands and Madagascar about 6.5 million years ago and have adapted to the many habitats available there. They used to be common on mainland Africa but natural selection, notably competition with other primates, has eliminated them from the continent.

There are almost 2 million species on Earth that have been catalogued and given a scientific name, the biggest number being insects. However, there are many more species as yet unidentified, and it is impossible to know exactly how many there are in the biosphere: 5 million? 10 million? 20 million? Even more overwhelming is trying to imagine how many species there were in the past that have now gone extinct. The organisms on Earth today represent much less than 1% of all life forms that have ever existed. How life has changed over time and how we make sense of the living world around us is the focus of this chapter. Understanding the mechanisms by which species evolve by natural selection is arguably one of the most important and influential concepts in biology. So much can be explained by natural selection, from why zebras have stripes, to why new bacterial populations that are resistant to antibiotics are being found in hospitals.

5.1 Evidence for evolution

Understandings:

- Evolution occurs when heritable characteristics of a species change.
- The fossil record provides evidence for evolution.
- Selective breeding of domesticated animals shows that artificial selection can cause evolution.
- Evolution of homologous structures by adaptive radiation explains similarities in structure when there are differences in function.
- Populations of a species can gradually diverge into separate species by evolution.
- Continuous variation across the geographical range of related populations matches the concept of gradual divergence.

Applications and skills:

- Application: Development of melanistic insects in polluted areas.
- Application: Comparison of the pentadactyl limb of mammals, birds, amphibians, and reptiles with different methods of locomotion.

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Looking for patterns, trends, and discrepancies: there are common features in the bone structure of vertebrate limbs despite their varied use.

Charles Darwin (1809–82).

Darwin and Wallace

At the age of 22, Charles Darwin had the opportunity to travel on board the HMS *Beagle* for a scientific exploration mission starting in 1831 and lasting for 5 years. Little did he know that it would allow him to see nature in a new way and come up with what would become one of the most important, controversial, and misinterpreted ideas in biology: evolution by natural selection.

Darwin was not the only person to develop a theory to explain evolution. Darwin was surprised to discover in 1858 that Alfred Russel Wallace had independently developed a nearly identical theory. The two men presented their ideas jointly to the Linnaean Society in 1858.

What is evolution?

Evolution is defined as the process of cumulative change in the heritable characteristics of a population. The word heritable means that the changes must be passed on genetically from one generation to the next, which implies that evolution does not happen overnight. The word cumulative is in the definition to stress the fact that one change is usually not enough to have a major impact on a species. Finally, the word population is in the definition because the changes do not affect just one individual.

Over time, if enough changes occur in a population, a new species can arise in a process called speciation. The members of the new population will be different enough from the pre-existing population that they came from that they will no longer be able to interbreed. Such a process is rarely observable during a human lifetime. However, once you begin to understand evolution, it should become clear that all of life on Earth is unified by its common origins.

It has been argued that once evolution by natural selection is understood, many of the mysteries of nature are revealed. Although there are others, we will examine three phenomena that provide evidence for evolution by natural selection: the fossil record, animal breeding and homologous structures. Later, we will also look at DNA evidence. When the role of DNA in inheritance (genetics) became understood, it appeared to some to contradict evolution by natural selection; such contradictions often arise with new developments in science. In fact, DNA evidence provides new support for natural selection beyond anything Darwin could have dreamt of, and is referred to as the modern synthesis or neo-Darwinism, a combination of Darwin's ideas with a newer one, the idea of genetics that Mendel started, that was only confirmed long after both men had died.

The fossil record and evolution

It is impossible to travel back in time, and the best clues scientists have about what life was like thousands or millions of years ago come from fossils. Fossils are the petrified remains or traces of animals and plants, and the fossil record is the accumulation of evidence from these remains and traces, such as skeletons and footprints. Palaeontologists have been collecting and classifying fossils in an organized fashion for almost two centuries.

Fossil hunting is the job of palaeontologists, and the best palaeontologists are willing to travel around the globe searching for bones, footprints, and plant remains. Some countries have policies controlling fossils to make sure that scientifically significant fossils are kept in museums or university collections. Other countries do not have such policies (or the policies are ignored by smugglers), and fossil hunters can sell fossils for profit to people wanting to add them to their personal collections. Should fossils be protected and conserved, or should they be considered as a commodity that can be bought and sold? What international organization should decide on and enforce such policies?

The Museum of Comparative Anatomy in Paris, France.

If you have ever been to a museum full of fossils classified by their age, you may have noticed a few things that palaeontologists have discovered that provide convincing evidence for Earth's evolutionary past.

- Overall, the life that existed more than 500 million years ago was vastly different in appearance from life today.
- Although planet Earth has had extensive oceans for most of its existence, fish fossils have only been found in rocks 500 million years old or younger (less than 15% of the 3.5 billion year existence of life on our planet).
- Although most of the top predators today are mammals such as bears, orcas, big cats, and wolves, none of them existed at the time of the dinosaurs or before.
- Apart from organisms such as certain types of sharks, cockroaches, and ferns, the majority of living organisms today have no similar form in the fossil record.

One conclusion that can be drawn from studying fossils is that life on Earth is constantly changing. However, most of the changes have occurred over huge timescales (hundreds of thousands or millions of years); timescales that humans find difficult to grasp.

Ageing fossils

The age of a rock can be determined by carefully examining differences in the ratios of isotopes. Isotopes are versions of atoms that are heavier or lighter than other versions of the same atom (carbon-14 has more mass than carbon-12). If a fossil of a bone or shell has a high level of carbon-14, for example, it is younger than a bone or shell that has a very low level of carbon-14. This is because carbon-14, also written ^{14}C, is

radioactive but slowly loses its radioactivity; as it gives off its radioactivity, it changes its identity into another atom, nitrogen-14. This process of a radioactive parent isotope changing into a stable daughter isotope is called decay. The speed at which this happens is expressed as an isotope's half-life. Half-life is defined as the time it takes for half of the parent isotope to decay into a stable daughter isotope.

The half-life of ^{14}C is 5730 years, meaning that, when an animal dies, its bones will have lost half their ^{14}C after 5730 years. After 11 460 years, half of that amount (now 25% of the original amount) will have decayed. Why is this important? Because by looking at the ratio of radioactive ^{14}C to stable ^{14}N, it is possible to determine the age of a fossil. If there is 12.5% of the radioactive isotope and 87.5% of the stable isotope, that means that three half-lives have gone by and the fossil is 17 190 years old. After a certain number of half-lives, there are so few ^{14}C atoms left that it is difficult to determine the age of the fossil with any accuracy.

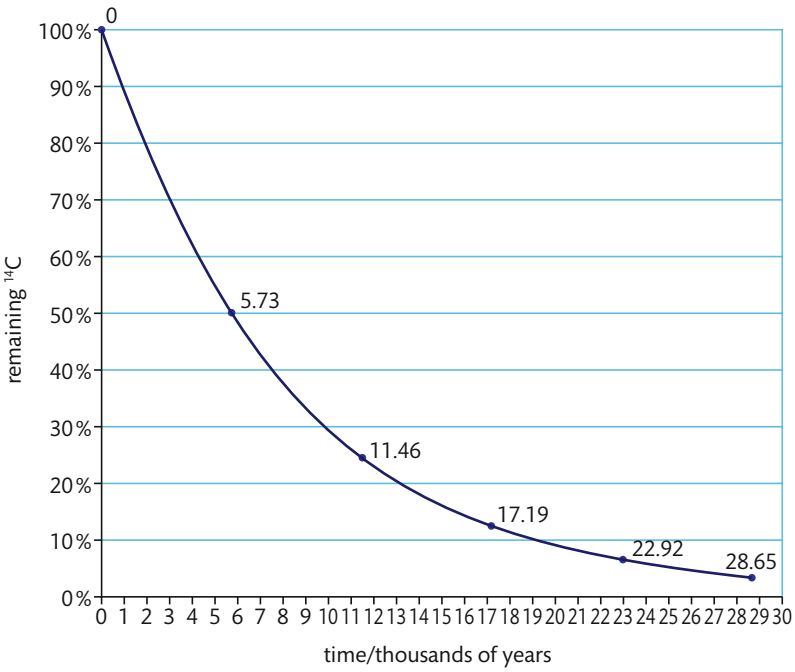


Figure 5.1 The effect of time on the proportion of radioisotope present in material containing carbon-14. The numbers on the curve show the passage of time (in thousands of years) through each successive half-life.

Fortunately, if there is insufficient ^{14}C, there are other radioactive isotopes that have much longer half-lives, such as ^{40}K (potassium-40). When the minerals in rocks crystallize from magma, they contain a certain percentage of ^{40}K ions. Once the minerals have hardened and crystallized, no more ^{40}K ions can be added. However, the number reduces as the radioisotope decays into more stable forms. Just as with ^{14}C, ^{40}K radiometric dating can be a useful tool in determining the age of a sample studied in a laboratory. Radiometric techniques with ^{40}K can be used to measure the age of rocks that formed from magma or lava between 100 000 years and 4.6 billion years ago.

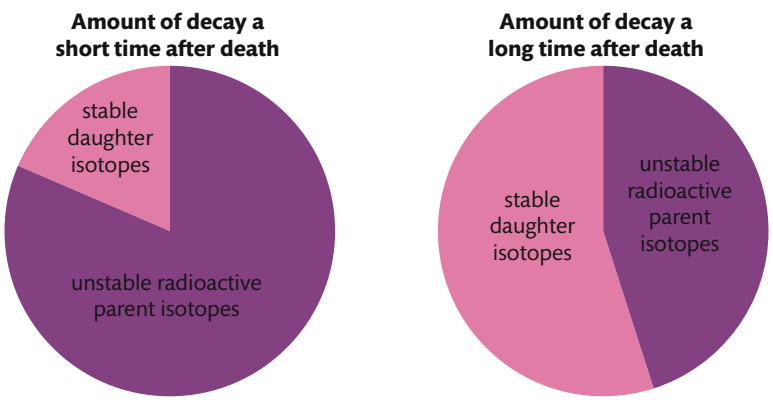


Figure 5.2 The proportions of radioisotopes and stable daughter isotopes in a once-living organism indicate the passage of time since the organism died. The higher the proportion of stable daughter isotopes, the older the fossil.

Artificial selection and evolution

The fossil record is far from complete, but the science of breeding domesticated animals, for example cattle, horses, dogs, sheep, and pigeons, provides a good record of recent changes in heritable characteristics.

By watching which males mate with which females, animal breeders can see which characteristics the offspring will have. Of the offspring produced, not all will be equally valuable in the eyes of a breeder. Some cows produce better milk, other cows produce better meat; one breeder may be interested in better milk, another in better meat. Over the years, breeders have learned to choose the males and females with the most desirable genetic characteristics and breed them together.

This cow has been bred to have a straight back for easier birthing and long legs for better milking by mechanical pumps. She is a product of artificial selection by humans and she never existed in this form before human intervention.

After practising selective breeding for dozens and sometimes hundreds of generations, farmers and breeders realized that certain varieties of animals now had unique combinations of characteristics that did not exist before. Today, the meat or milk available to us is very different from that which was produced a few generations ago, thanks to the accumulation of small changes in the genetic characteristics of livestock chosen by breeders.

Although this is evidence that evolution is happening as a result of an accumulation of small changes over time, the driving force is, of course, human choice. The farmers and breeders choose which animals will reproduce and which will not. This is called artificial selection and it should be obvious that it is certainly not the driving force of evolution in natural ecosystems.

Evolution of homologous structures by adaptive radiation

Other evidence for evolution comes in the form of homologous anatomical structures, which are similar in form but which are found in seemingly dissimilar species. One of the most striking examples of this is the five-fingered limb found in animals as diverse as humans, whales, and bats. Such limbs are called pentadactyl limbs because 'penta' means five and 'dactyl' refers to fingers. Although the shape and number of the bones may vary, the general format is the same, despite the fact that the specific functions of the limbs may be very different. Darwin explained that homologous structures were

This is the front right fin of a southern right whale showing five articulated fingers..

not just a coincidence but evidence that the organisms in question have a common ancestor.

They may be of different sizes, and show varied morphology (shape), but the basic shape and position of the limb bones are the same. This would suggest that all five-fingered organisms have a common ancestor.

Whales, for example, could probably swim just as well with a different number of fingers in their front fins, so the fact that there are five suggests that there is a reason other than swimming efficiency: that of a common ancestry with other five-fingered organisms.

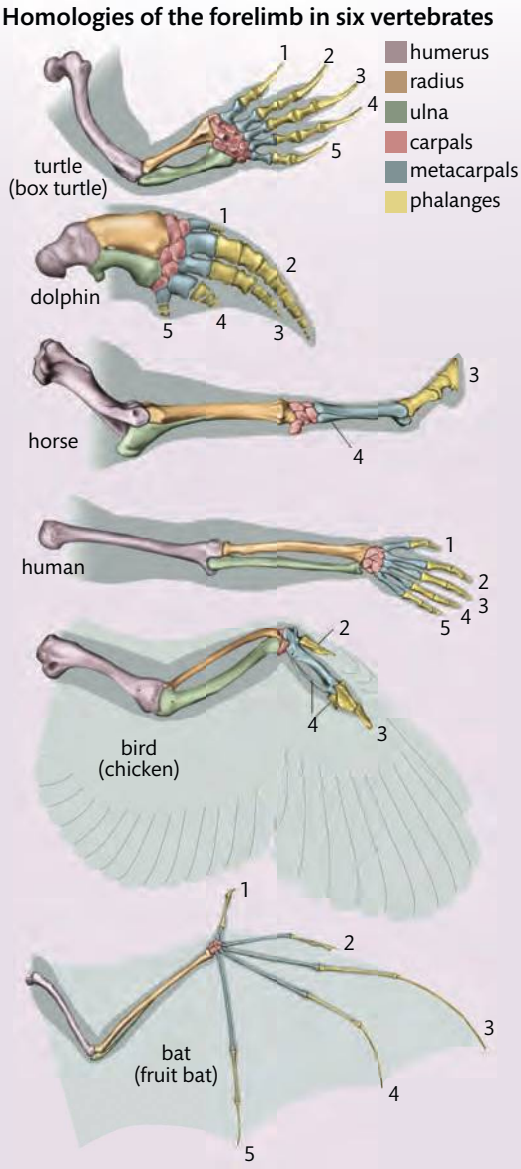


Figure 5.3 Pentadactyl forelimbs from various animals.

CHALLENGE YOURSELF

1 (a) Look at Figure 5.3 and complete Table 5.1.

Table 5.1

Characteristic	Bat	Bird	Human	Horse	Dolphin	Turtle
Number of digits (fingers)						
Description of phalanges (finger bones) (short/long, wide/narrow)						
Type of locomotion that the limb is best adapted for						

(b) There are two animals in Table 5.1 that have reduced their number of digits over the course of evolution. For these two animals, explain why it would have been a disadvantage to have kept all 5 digits. Limit your answer to the type of locomotion.

(c) Compare and contrast the salamander's forelimbs (Figure 5.4) to the organisms in Table 5.1. Be sure to address the idea of number of digits and locomotion.

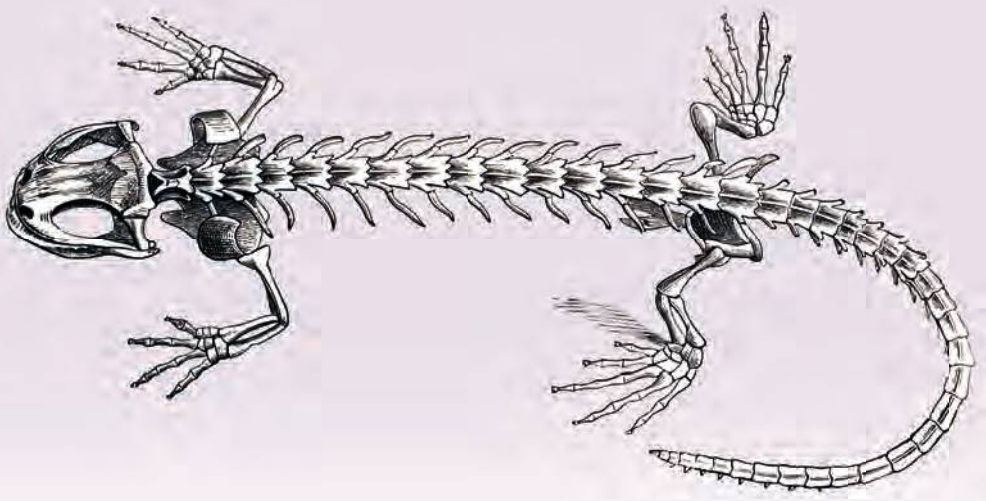


Figure 5.4 A salamander skeleton.

Species divergence

The process of an evolving population changing significantly enough so that the production of offspring with the original population becomes impossible is called speciation. In short, two populations of a species have diverged (separated), and a new species has evolved from an old one; both species will then continue on their separate ways.

Recall that a species must be able to freely interbreed with members of the same species to produce fertile offspring. If there has been a significant enough difference in two separated populations and they can no longer interbreed, a speciation has occurred.

Adaptive radiation

Adaptive radiation occurs when many similar but distinct species evolve relatively rapidly from a single species or from a small number of species. This happens as variations within a population allow certain members to exploit a slightly different niche in a more successful way. A niche is a position or role within a community of an ecosystem. By natural selection and the presence of some kind of barrier, a new species can evolve. A barrier separating populations might be a mountain range or a body of water.

An example of this are the primates found in Madagascar and the Comoro Islands off the south-east coast of Africa. Millions of years ago, without competition from monkeys or apes, lemurs on these islands were able to proliferate. Large numbers of offspring meant a greater chance for diversity.

Among the wide range of variation in lemur species, some are better adapted for living on the ground instead of in trees. Others are better adapted for living in lush rainforests, while some can survive in the desert. Most lemurs are active during the day (diurnal) but some are nocturnal. The reason why there are so many different species of lemur with different specialties is because of adaptive radiation.

Lemurs are primates found in Madagascar. They are a good example of adaptive radiation.

Not a single species of living lemur has been found anywhere else in the world. And yet fossils of their ancestors have been found on the continents of Africa, Europe, and Asia. What happened? It is believed that lemurs were not successful in competing with apes and monkeys, because as soon as traces of the latter start to become more prevalent in the fossil record, the lemur-like organisms become rare.

This would explain why continents and islands tend to have either prosimians (such as lemurs) or anthropoids (such as monkeys and apes), but not both types of primate. This is being confirmed today because more than a dozen species of lemur have become extinct recently, and many more are endangered, as a result of the activities of the most recently evolved anthropoid: humans.

Other examples of adaptive radiation can be seen in birds such as Darwin's finches (described in Section 5.2) on the Galapagos Islands and the Hawaiian honeycreepers. The honeycreepers have a wide variety of beak shapes, some of which are adapted exclusively to sip the nectar of flowers found only on Hawaii. It is believed that all the Hawaiian honeycreepers are the result of the adaptive radiation of a few members of one species that arrived on the islands.

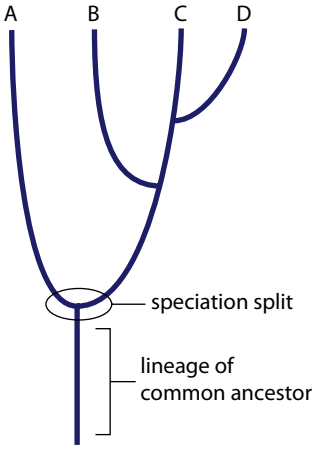


Figure 5.5 Speciation split shown on a phylogenetic tree.

Continuous variation and the concept of gradual divergence

In Figure 5.5, species A, B, C, and D come from a common ancestor. If any two of the species tried to mate, they would not successfully produce fertile offspring.

Figure 5.6 illustrates how one species can have various splits over time, creating a greater diversity between species. In some cases, the branches of the phylogenetic tree can become spaced so far apart that the species, although once closely related, do not physically resemble each other anymore. For example, when comparing a bird that has a long, thin beak to another with a short, fat beak, it is difficult to imagine that they are both descendants from the same species. And yet biologists have observed this in many species, notably ones that are spread over a wide geographical area.

From the example of the saltmarsh grass in the Nature of Science box, it is possible to see that, within a species that has a wide geographical distribution, there can be measurable differences in DNA. This is because the climate and soil are different in different locations. As a result, the populations adapt to the conditions available to them, and some versions of genes will be selected for and others will be selected against so that the populations are best adapted to their areas. This is called selective pressure. If this phenomenon continues to produce genetic differences over a long enough time, it is not difficult to imagine a point at which the differences between two separated populations are so great that they no longer belong to the same species. There comes a tipping point beyond which the differences outweigh the similarities and the two populations in question can no longer freely reproduce together. For example, if pollen from a northern species of marsh grass was used to pollinate flowers from a southern population, and no seeds or fertile offspring were produced, a speciation would have taken place.

NATURE OF SCIENCE

There is a species of plant that grows in coastal saltwater marshes called saltmarsh cordgrass, *Spartina alterniflora*. It plays an important role in providing habitat for organisms both above and below the water. The following investigation was carried out to determine whether differences in this plant along the eastern coast of the USA were the result of genetic variations or not. To test this, a group of scientists, led by Denise Seliskar, took samples of the cordgrass from three different states from different latitudes:

- Massachusetts (41° 34′ N)
- Delaware (38° 47′ N)
- Georgia (31° 25′ N).

They grew the plants in the same location at a research facility in Delaware and compared their growth in Delaware with how these plants grow in their native habitats. Notice how only one population is growing in its native state: the one from Delaware. The others have been moved either north or south of their native state. The investigators measured the growth of the plants over a 5-year period in various ways, including:

- biomass (how much dry organic material is produced in a year)
- height
- stem diameter.

The hypothesis was that, if there is no genetic variation within this species, then the three populations of plants from different latitudes will have similar growth patterns when grown in Delaware, because they are all given the same growing conditions of soil, water, light, and temperature.

The results, published in the *Journal of Ecology*, February 2002, were as follows. The population that originated from the south (Georgia) grew the most robustly. It showed the greatest biomass, height, and stem diameter. This is typical of plant growth in populations in southern latitudes where the climate is warmer. The northern-most population showed the least robust growth, matching values that were recorded in populations of its native Massachusetts. The population originally from Delaware showed no significant difference in growth from other populations in Delaware.

What can be concluded from this? Before you read on, can you reach your own conclusion? Look back at the hypothesis and decide if the data confirm or refute it.

Answer: the difference in growth refutes the hypothesis. The plants showed growth patterns similar to their native locations, suggesting that their DNA has a significant influence on their growth. The DNA imported from the southern latitude instructed the plants to grow larger, the DNA imported from the northern latitude instructed the plants to grow smaller. This indicates that there is variation in genetics from one geographical location to another.

This may not be the only explanation; perhaps there are others. However, in science, generally the principle of parsimony is applied: we look for the simplest, least convoluted explanation. For example, if we wanted to introduce the idea that an extra-terrestrial visitor came down to the experimental marsh where the plants were growing in order to somehow influence their growth with a special ray gun, we could. But that would not be parsimonious: it would be convoluted and would not be scientific because there is no evidence for it.

When scientific investigations are completed, usually they generate new questions or new ideas for further investigation. What do you think the investigators of the cordgrass would like to find out next?

Transient polymorphism

Within a population there is often more than one common form. Different versions of a species are referred to as polymorphisms (meaning many shapes) and can be the result of a mutation. One example of such an organism is *Biston betularia*, the peppered moth, which lives in temperate climates.

This species of moth can have a peppered (grey) form or a melanic (black) form; the melanic form is a rare mutation that usually affects less than 1% of a population. The grey form is well camouflaged against light-coloured surfaces, such as tree branches covered with lichens. One of the reasons why they are much more numerous in the population is that black moths are seen more easily against light-coloured lichens and thus are more frequently preyed upon by birds.

On close examination, you should be able to see two moths on the tree trunk covered in lichen.

Around the time of Darwin (1860s), a phenomenon was underway that continued for over a century: the industrial revolution. The melanic form of the peppered moth, called *carbonaria*, was increasing in number. Lichens, like the ones pictured on the tree in the photo, are very sensitive to air pollution, and the industrial revolution was producing chemicals, such as sulfur dioxide, that kill lichens. In addition, the air was filled with black soot from the large quantities of coal being burnt. As a result of this, the lichen-free, soot-darkened branches were a more difficult place for light-coloured peppered moths to hide: their camouflage simply did not work anymore. Birds eat moths and visual predation is facilitated when camouflage is poorly adapted.

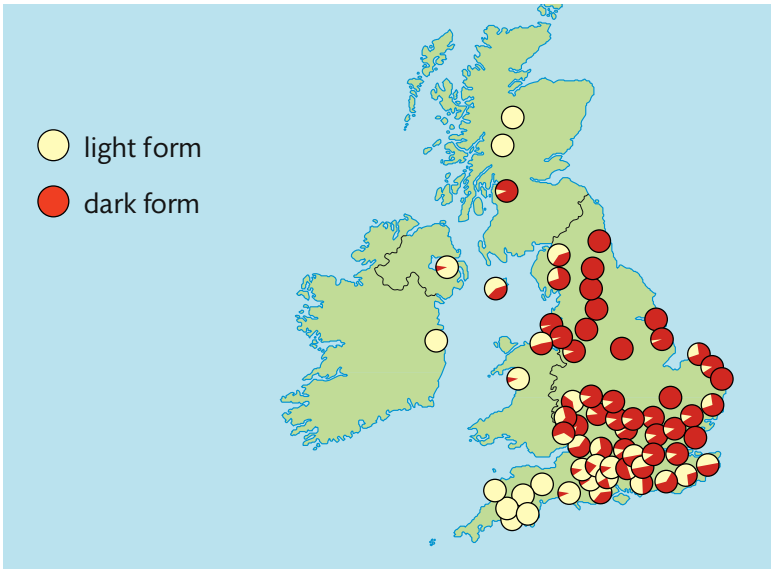


Figure 5.6 A map of the distribution of light-coloured and dark-coloured peppered moths in Great Britain under the influence of industrial pollution.

In places near industrial centres, the *carbonaria* moths accounted for 95–100% of all the peppered moths observed. Today, the percentages of *carbonaria* in a population rarely go above 30% and are often 0%. This is because of a significant improvement in air quality thanks to measures such as the UK Clean Air Act of 1956. These changes in the peppered moth population over time, from light-coloured to dark-coloured and then back again, is an example of transient polymorphism, temporary changes in the form of a species.

Worked example

Using the map in Figure 5.6 and the information presented about peppered moths during and after the industrial revolution in the UK, answer the following questions.

1 Statistics for peppered moths in the 1700s do not exist. Predict what the percentage of peppered moths would have been a century before Darwin lived, before the effects of the industrial revolution on trees.

2 **(a)** In the 1700s in a relatively non-polluted area where lichen is still growing on trees and soot is not a problem, a flock of birds comes to an area where there is a large number of grey peppered moths and only a very small number of black peppered moths. Explain why it is the black ones that have a higher chance of being eaten.

(b) What influence does this have on the population of dark-coloured moths?

3 Many decades later, the pollution has taken its toll on the lichen, and the soot in the air has blackened trees near industrial areas. Now when a flock of birds arrive to eat the moths, which kind gets eaten and why?

4 **(a)** Explain how it is possible that, by the 1900s, when the map in Figure 5.6 was made, most of the moths were dark-coloured.

(b) Explain how it is possible that now, in the 2000s, the population is back to being light-coloured.

Solutions

1 Because the mutation for melanism is very rare, it would be expected that the percentage of dark-coloured moths would be very low, certainly less than 10% and probably closer to 1%.

2 **(a)** The black ones will be eaten because they are easy to spot against a light background.

(b) This keeps the population of mutated dark moths at very low levels.

3 Now that the background colour has changed, the light-coloured moths will get eaten. This is because they are no longer able to hide against the darkened background.

4 **(a)** Because they were able to escape being eaten by birds, *carbonaria* moths were able to survive and pass on their genes to the next generation, something that was not possible before. In contrast, because the light-coloured moths were being spotted and eaten, they could no longer pass on their genes to the next generation. Over many generations, this process reduced the number of light-coloured genes from the population and favoured the allele for dark coloration. The same process happened for dozens of other species of moth.

(b) Ever since the Clean Air Act was passed in 1956, air quality around industrial zones of the UK has improved: there are fewer sulfur dioxides and less soot in the air. This has allowed the pollution-sensitive lichen population to return and allowed the bark on tree trunks and branches to return to their non-blackened colour. Now that the light-coloured moths can hide better and avoid being eaten, their numbers have increased. In contrast, *carbonaria* moths are no longer effectively camouflaged and get spotted and eaten by birds, reducing their presence in the population.

TOK

Is the peppered moth a good example of evolution? The story of the peppered moth is a long one, involving many ups and downs. The data have been criticized, questions have been raised about whether bird predation is the only reason for the population change, and most of the photos of moths trying to rest or hide on tree trunks have been revealed as being staged: they are of dead moths stuck to the trunks for the purpose of the photo. Also, the idea of industrial melanism has been criticized as an example of evolution because no new species is formed: we started with a peppered moth and we finished with a peppered moth.

Although it is one of the most cited examples of modern evolution by natural selection, it has been suggested by some critics that it should be removed from textbooks because it is not a valid example and is based on sloppy science. Research this debate and trace the story's ups and downs. What are the arguments for and against the peppered moth as an example of evolution by natural selection? Should it continue to be used in classrooms as an illustration of how evolution works? When there are disagreeing sides, which one should we believe? What have you learned in Theory of Knowledge to help you to make your decision?

TOK

Trying to find out what happened in the past is the job of both historians and evolutionary biologists. Do they use the same methods to infer and deduce what the past was like? What counts as knowledge for an evolutionary biologist, and how is that similar or different from what counts as knowledge for a historian?

Natural scientists often use experimentation in laboratories to test out their hypotheses. And yet, it is impossible to carry out investigations such as breeding experiments with organisms that have gone extinct. How is the scientific method different for a scientist who studies fossils and evolution compared with a scientist who studies genetic traits in contemporary organisms?

One of the most energetic proponents of neo-Darwinian ideas is the evolutionary biologist Richard Dawkins. In his writing, he points out the difficulty of applying the term species to organisms that lived in the past. For example, he asks his readers to picture a modern-day rabbit and imagine the rabbit's parents. There is no doubt that both of the parents and the offspring are all three of the same species, despite the fact that the offspring is not identical to its parents. We could probably be safe in taking this thought experiment back many generations and assume that, even though there are variations in each generation, there comes a time when the ancestor was significantly different from the modern rabbit. But how far do we go? It is difficult to know how many thousands of generations in the past we would need to study in order to declare that, at that point, that ancestor was, in fact, a different species.

Exercises

1. Define the term evolution.
2. Concerning species on Earth, describe two overall trends that can be seen in the fossil record.
3. Explain how selective breeding can be a good example of evolution by selection, even though it is not natural selection.
4. List two examples of adaptive radiation.

NATURE OF SCIENCE

Use theories to explain natural phenomena: the theory of evolution by natural selection can explain the development of antibiotic resistance in bacteria.

5.2 Natural selection

Understandings:

- Natural selection can only occur if there is variation amongst members of the same species.
- Mutation, meiosis, and sexual reproduction cause variation between individuals in a species.
- Adaptations are characteristics that make an individual suited to its environment and way of life.
- Species tend to produce more offspring than the environment can support.
- Individuals that are better adapted tend to survive and produce more offspring while the less well adapted tend to die or produce fewer offspring.
- Individuals that reproduce pass on characteristics to their offspring.
- Natural selection increases the frequency of characteristics that make individuals better adapted and decreases the frequency of other characteristics, leading to changes within the species.

Applications and skills:

- Application: Changes in beaks of finches on Daphne Major.
- Application: Evolution of antibiotic resistance in bacteria.

Guidance

- *Students should be clear that characteristics acquired during the lifetime of an individual are not heritable. The term Lamarckism is not required.*

The mechanism for evolution

Besides providing evidence for evolution, Darwin and Wallace suggested a mechanism for evolution: natural selection. How does this work? It all starts with the overproduction of offspring and the presence of natural variation in the population; then there is a struggle between competing varieties that leads to survival for some and death for others. This section will look at how evolution works through natural selection.

Variation within populations

Organisms such as bacteria reproduce simply by making a copy of their genetic information and then splitting into two using the process of binary fission. The result is that the second generation is identical to the first. In fact, many future generations will be identical or show very little change. There is little chance for the DNA to be modified.

The story is very different for species that reproduce sexually. When a cat has kittens, for example, each one is slightly different, or when a population of guinea pigs interbreeds there can be a wide variety of offspring.

Variation and success

Variation is closely related to how successful an organism is. A baby bird that has pigments that give it a colour matching its surroundings will have a better chance of not being seen by a predator. A fish with a slightly different shaped mouth might be able to feed from parts of a coral reef that other fish are not able to access. A plant that produces a different shaped flower might have a better chance of attracting insects for pollination.

It might seem obvious that a young bird with a colour that makes it very conspicuous to predators has little chance of surviving to adulthood. On the other hand, it might be more attractive to mates. A fish with an oddly shaped mouth may, in fact, be incapable of feeding adequately and die of starvation. A plant that produces flowers that are not attractive to insects will not have its flowers pollinated and will not produce any offspring.

As we have seen with the peppered moth, how frequent an allele is can change over time because of changes in the environment. This is only possible if there is more than one form of the allele. If the peppered moth did not have a mutation giving some members a dark colour, it is possible that certain populations would have been completely wiped out when their camouflage no longer worked

Variation can be seen in this population of guinea pigs.

against a dark background. In contrast, in bacteria, for example, there are essentially no differences within a population: all members of the population are genetically identical copies of each other. This means that if an adverse change happened in the environment, such as a change in pH, if one bacterium is susceptible to the change in pH and dies, they in fact all die because they all have the same vulnerability. In species where there is variation, a change in the environment will eliminate some but not all members of the population. This is why variation is a strength and not a weakness in a population. We will see how this works as this section continues.

The idea of eugenics is that, if human breeding is controlled, it could improve the population by favouring desirable characteristics and eliminating undesirable ones. This is highly controversial, and historical applications of it have been widely criticized. Trying to breed a 'superior race' where everyone has the same characteristics is contradictory to the concept Darwinian evolution is based on: variety. The resilience of a species is highly dependent on variety.

Mutation, meiosis, and sexual reproduction

There are three main mechanisms that give organisms in a species their variation:

- mutations in DNA
- meiosis
- sexual reproduction.

Mutation

Mutations can sometimes produce genes that lead to genetic diseases, and can have devastating effects on the survival of some individuals in a species. However, sometimes a mutation can produce a characteristic that is advantageous, perhaps a slightly faster growth rate for a tree or better frost resistance for a plant. A beneficial mutation for a bird or insect might result in a different camouflage that better matches a changing habitat. In each generation, only a few genes mutate, and most mutations produce effects that are neither useful nor harmful. As a result, sexual reproduction is a much more powerful source of variation in a population because thousands of genes are mixed and combined. But sexual reproduction is only possible thanks to meiosis.

Meiosis

Meiosis, you will recall from Section 3.3, enables the production of haploid cells to make gametes (sperm cells and egg cells). At the end of meiosis, four cells are produced that are genetically different from each other and only contain 50% of the parent cell's genome. An individual that reproduces sexually can produce huge numbers of possible combinations of half the genetic material it possesses, thanks to meiosis. For example, in a woman's lifetime, it is nearly impossible for her to produce the same egg twice. This is why, no matter how many pregnancies she has, she will never have the same child twice from two different pregnancies. The only way identical humans have ever been formed is when two embryos are formed from a single egg, i.e. identical twins, and even then there are slight genetic differences between the siblings.

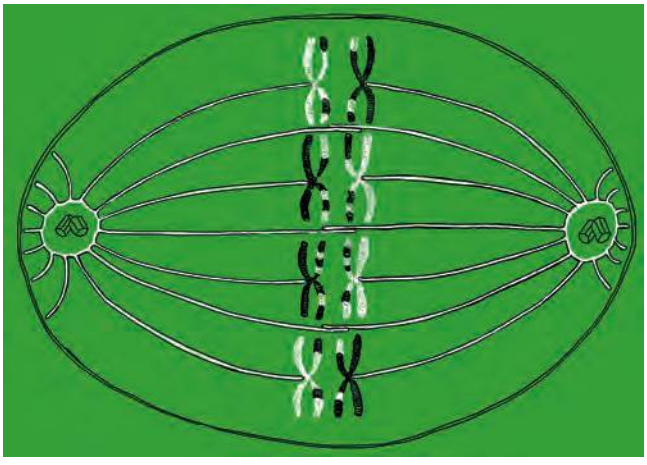


Figure 5.7 Random orientation during metaphase I and crossing-over (shown by banding on sister chromatids) promote variety in the gametes. Each sister chromatid will separate into separate haploid cells at the end of meiosis (see Section 3.3).

The variety in gametes comes mainly from the process of random orientation during metaphase I. The lining up of chromosomes in a random order is like shuffling a deck of cards, and it greatly promotes variety in the egg cells or sperm cells produced. In addition to this, the process of crossing-over contributes to the shuffling of genetic material and further increases the genetic variety.

Sexual reproduction

As we have seen, asexual reproduction such as binary fission in single-celled organisms does not promote variety in the population. Generally speaking, in an asexually reproducing population, all the members of the population are identical. There may be rare exceptions of mutations or gene transfer, but overall such populations can remain identical generation after generation. The consequence for this is that natural selection only leaves two choices for the population: survive or die. One of the causes of the Great Famine in Ireland in the mid-1800s was that the potatoes had been produced asexually and were all clones, making them all susceptible to the same infection by a microorganism that causes potato blight. This also illustrates that if there is no variety in a population, there is a very limited number of outcomes: the whole population either survives or dies. This is why variety is so important to natural selection. More possibilities lead to more possible outcomes: some members of the population survive without any adverse effects, others may be affected in a negative way but still survive, and others may die. Variety in the population allows some individuals to be better adapted to whatever change in the environment is harmful to others.

Part of what determines whether or not a female animal becomes pregnant is that all the conditions must be right inside her body, and that sperm cells must be present at the opportune moment when an egg is ready. Of the many sperm cells that may be present, only one will penetrate the egg. In determining exactly which sperm cell and egg will meet and fuse together, a certain amount of chance and luck are involved. In non-human primate species, such as chimpanzees, for example, when a female is fertile, many males may copulate with her to try to impregnate her. In such a scenario, it is impossible to guess which male's sperm cells will successfully fertilize her egg. It is largely up to chance. In flowering plants, which bees will land on which flower of a population, with what pollen from another flower in that population, is also a matter of chance.

Which of these yellow pollen grains on the bee's body will pollinate the next flower it visits?

There are three main sources for variation in a population:

- **mutations in DNA**
- **meiosis**
- **sexual reproduction.**

But make no mistake, although these two mechanisms for increasing variety (meiosis and sexual reproduction) rely on chance, it would be unfair to conclude that all of life is just a game of chance. As we will see, natural selection has another side to it that has little to do with chance and allows for systematic accumulations of small changes to produce highly adapted forms of life.

To adapt or not to adapt?

Although it is possible for some organisms to adapt to changes in their environment within their lifetimes, this is not the kind of adaptation referred to in evolution. For example, just because an individual hare can shed its brown fur and grow white fur for the winter in order to be better camouflaged against the snow, does not mean that the individual has 'evolved' from one season to the next. Evolution happens to populations and its effects are only visible over many generations.

The adjective adaptation and the verb to adapt are freely used when talking about evolution. However, the terms have very precise meanings within the framework of natural selection and should not be confused with other uses of the term, notably for human behaviour. For example, humans can consciously decide to adapt to a situation: think of a student learning the language of a country he or she has just moved to, or of a person who is used to driving his or her car on the right-hand side of the road and rents a vehicle in a country where driving is done on the left-hand side and so adapts very quickly to left-hand driving. These are conscious adaptations made by individuals. In nature, the vast majority of adaptations referred to in evolution and natural selection are unconscious adaptations made by populations rather than by individuals.

One example we have already seen is the adaptation of the peppered moth populations over time before and after the industrial revolution. On light-coloured backgrounds, the grey moths were better adapted, whereas on dark-coloured backgrounds, the black moths were better adapted. Another example is that a giraffe's neck is well adapted for reaching leaves high up in trees. If a giraffe was born with a mutation that gave it a short neck, it would have trouble competing with other giraffes to get leaves. A short neck is an example of a characteristic that is not well adapted for a giraffe's lifestyle.

An organism that has characteristics that are well adapted for its environment is said to be fit. The characteristics it possesses fit well into its environment.

Natural selection tends to eliminate from a population individuals that show low fitness, whereas the fittest individuals in a population have a higher likelihood of surviving. Although there are rare exceptions, individuals are usually incapable of changing themselves to adapt. For example, a giraffe born with a short neck cannot stretch its neck to get a longer one. Rather, because it will have difficulty feeding itself and surviving, the chances are very low that it will find a mate and reproduce to be able to pass on its genes to the next generation. Hence the alleles for making a short neck are not found in the giraffe population.

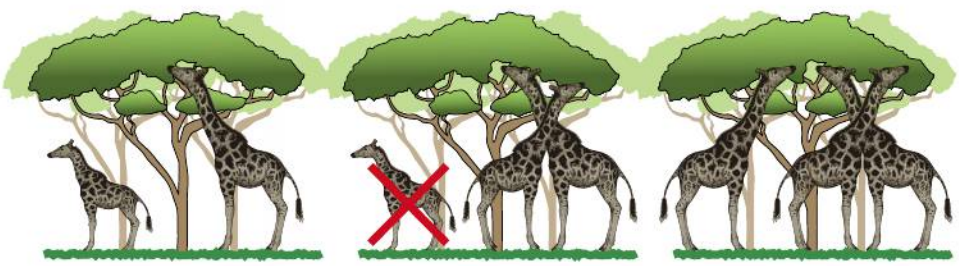


Figure 5.8 The giraffe's long neck explained by natural selection.

Ancient population with variation in neck lengths. Giraffes with longer necks can reach more food and have a better chance of survival. Those born with shorter necks find less food and have lower chances of survival.

After many generations, the genes for longer necks are passed down more successfully than the genes for shorter necks. The population sees more and more long-necked giraffes and fewer and fewer short-necked giraffes until they all have long necks.

Too many offspring

Darwin noticed that plants and animals produce far more offspring than could ever survive. Plants often produce hundreds or thousands more seeds than necessary to propagate the species. Mushrooms produce millions more spores than ever grow into new mushrooms. A female fish lays hundreds or thousands of eggs but only a handful survive to adulthood.

This seems paradoxical, because the production of seeds, spores, and eggs involves using energy and nutrients that also are vital to the parents' survival. Why are such valuable resources squandered on so many excess cells that are never going to give rise to viable offspring? The answer is to maximize the chances of some offspring surviving, even if the survival rate is less than 1%.

Having too many offspring and not enough resources is a problem of supply and demand. There is high demand for water, space, nutrients, and sunlight, but there is a limited supply. The consequence is competition for these resources in order to stay alive. This is called the struggle for survival.

Many species of animal are territorial and possessive of their food supplies: they spend a great deal of time and energy defending their resources. Trees, too, defend their resources, by having active compounds such as tannins and alkaloids in their trunks to ward off attackers such as insects. All these adaptations make it difficult for a new arrival to find enough resources. As a result, parents send out dozens, hundreds or thousands of potential offspring into the world. Parent organisms that do not produce as many may find the probability of their genes being passed on greatly reduced.

Adaption and survival

Evolution is not just based on chance. In a situation where there are too many organisms for limited resources, it is obvious that some individuals will succeed in accessing those resources and the rest will fail. In other words, there is a selection. Exactly which individuals survive and which ones do not is not based on chance alone but determined by their surroundings and the compatibility of their characteristics with those surroundings. The steps of evolution by natural selection are outlined below.

- Overproduction of offspring and, in those offspring, natural variation as a result of genetic differences (e.g. body size, morphology, pigmentation, visual acuity, resistance to disease). In the offspring:
  - useful variations allow some individuals to have a better chance of survival (e.g. hiding from predators, fleeing danger or finding food)
  - harmful variations make it difficult to survive (e.g. inappropriate colour for camouflage, heavy bones for birds, having such a big body size that there is not enough food to survive).
- Individuals with genetic characteristics that are poorly adapted for their environment tend to be less successful at accessing resources and have less chance of surviving to maturity.
- Individuals with genetic characteristics that are well adapted for their environment tend to be more successful at accessing resources and have a better chance of surviving to maturity. Such individuals are said to have better fitness.
- Because they survive to adulthood, the successful organisms have a better chance of reproducing and passing on their successful genetic characteristics to the next generation.

CHALLENGE YOURSELF

One quantitative study done over a 30-year period by Rosemary and Peter Grant showed differences in beak sizes of ground finches, *Geospiza fortis*, from two islands of the Galapagos: Daphne Major and Santa Cruz. You can learn more about this study through an online exercise including analysis of the data they collected. You can find a link to this activity in the hotlinks box at the end of this section.

- Over many generations, the accumulation of changes in the heritable characteristics of a population results in evolution: the gene pool has changed.

As you can see, it is impossible to sum up all these concepts in one catchy phrase such as 'the law of the jungle'. Although Darwin himself eventually adopted the phrase 'survival of the fittest', the idea of evolution by natural selection is more complex than that. In addition, many people have the misconception that what Darwin said was 'only the strongest survive'. This is simply not true.

The theory of evolution by natural selection is full of subtleties. This could be one of the reasons why it is so widely misunderstood by the general public. For example, an organism that is well adapted to its environment is not guaranteed success, it simply has a higher probability of survival than another that is less well adapted. Dinosaurs such as the sauropods were the biggest, strongest animals ever to walk the planet. But they did not survive the environmental changes that drove them to extinction. In fact, the fossil record indicates that more than 99.99% of all life that has ever existed on Earth is now extinct.

Plover eggs show adaptations that have been acquired by natural selection. The colour and spots help to camouflage them from predators.

In the photo of plover eggs, the colours and speckles act as effective camouflage, making these eggs difficult to spot by predators. Plover chicks are also speckled for camouflage. If a mutation caused a shell to be bright white and/or the chicks to be bright yellow, the mutation would be unlikely to be an advantage to this species. On the contrary, a white egg or yellow-bodied chick would attract the attention of a predator, the egg or chick would be eaten, and the possibility of passing on the mutation to the next generation would be zero.

It is crucial that you remember Darwin's steps of how natural selection leads to evolution. Be sure to memorize the following: (1) overproduction of offspring; (2) variation within the population, as a result of meiosis, sexual reproduction, and mutations; (3) struggle for survival, because there are not enough resources for all members of the population; (4) differential survival, those individuals best fit for their environment tend to survive better; and (5) reproduction, those who survive can pass on their genes to the next generation. It is through these steps that populations evolve. Remember that, even though the changes can be observed in individuals from generation to generation, what is of importance is what happens at the level of populations rather than at the individual level.

Passing on successful characteristics

It should be obvious that an individual that never reaches maturity will not be able to pass on its genes to the next generation. An individual that is poorly adapted to its environment, such as an insect with deformed mouthparts that make it impossible to feed, is not likely to survive to adulthood and be able to reproduce.

On the other hand, an individual showing high fitness has a better chance of surviving until adulthood and reaching maturity. Individuals that reach maturity have the possibility of reproducing and passing on their genetic material. Again, there is no

guarantee that fitness will allow survival or that survival will allow reproduction, but, in order to reproduce, one thing is certain: survival must come first. Remember the example of the giraffes: those who were born with the alleles to make necks long enough to access better food sources had a greater chance of surviving and passing on those alleles, whereas those with short-neck alleles had more trouble finding enough food and were less frequently able to survive to pass on their alleles.

Natural selection and the frequency of characteristics

Pesticide resistance in rats and multiple antibiotic resistance in bacteria are both carefully studied modern examples of natural selection. What is striking about these examples is their rapidity. Although evolution is generally considered to be a long-term process, the mechanism of natural selection can sometimes be quick, taking place over months, years or decades, rather than millennia. As you read the descriptions below, see if you can identify the main features of how natural selection works: variation in the population making some individuals better suited for their environment than others, overproduction of offspring leading to a struggle for survival, differentiated survival because some die and some live, and, finally, the passing on of successful traits to the next generation.

Pesticide resistance in rats

Pesticides are chemicals that kill animals that are regarded as pests. Farmers use them to eradicate pests, such as rats that eat their crops. Consider the following scenario.

1. Once applied in the fields, pesticides kill all the rats … or so the farmer thinks.
2. As a result of natural variation, a few rats from the population on the farm are slightly different and are not affected by the poison.
3. The resistant rats are better adapted to survive in the presence of the pesticides and now, thanks to the farmer's actions, have no other rats to compete with for a food supply. Hence, they thrive and reproduce, making a new population in which some or all of the members possess the genes that give resistance to the pesticide.
4. Seeing rats again, the farmer puts out more of the original poison; this time fewer rats die. Because the characteristic of poison resistance was favoured in the rat population, it is now much more common in the population.
5. To kill the resistant rats, a new pesticide must be used.

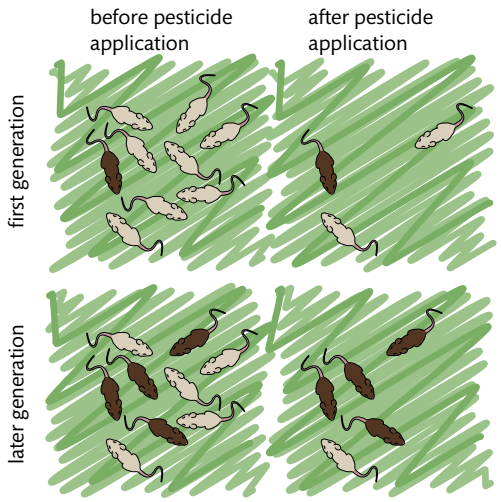


Figure 5.9 How populations of pests such as rats develop resistance by natural selection. Notice the difference in the number of resistant rats before the first pesticide application and after the application.

It is important to note that, in this example, we cannot say that the rats become immune to the poison. Although the term 'immunity' is sometimes interchangeable with the term 'resistance', that is not the case here. Immunity develops within the lifetime of an individual; pesticide resistance is a change that evolves in a population from one generation of rats to the next generation. The evolution happened in the population, not in any single rat. A rat is either born with a susceptibility to be killed by the pesticide or is born with resistance to it. An individual rat cannot adapt and evolve into a resistant rat.

It is also important to note that the characteristics that change and evolve over time must be heritable (passed on by genes). An example of this is that farmers have been cutting off the tails of sheep for many centuries and yet sheep continue to be born with long tails. In other words, characteristics acquired during an organism's lifetime cannot be passed on to the next generation and so do not have a part in the theory of evolution by natural selection.

Sheep are still born with long tails, despite being removed by farmers for countless generations.

Antibiotic resistance in bacteria

Antibiotics are medications such as penicillin that kill or inhibit the growth of bacteria. They are given to patients suffering from bacterial infections. They are also sometimes given to people who are suffering from something else and, because their immune system is weak, are at a greater risk of a bacterial infection. However, overuse of antibiotics can lead to the production of resistant strains of bacteria.

Antibiotic resistance in bacteria develops in several steps. Consider the following scenario.

1. A woman gets a bacterial infection such as tuberculosis.
2. Her doctor gives her an antibiotic to kill the bacteria.
3. She gets better because the bacteria are largely destroyed.
4. By a modification of its genetic makeup, however, one bacterium is resistant to the antibiotic.
5. That bacterium is not killed by the antibiotic and it later multiplies in the patient's body to make her sick again.

6 She goes back to the doctor and gets the same antibiotic.
7 This time, no result: she is still sick and asks her doctor what is wrong.
8 The doctor prescribes a different antibiotic that (hopefully) works. But if the population of bacteria continues to acquire mutations, new strains could show resistance to all the antibiotics available.

Because bacteria reproduce asexually, genetically they generally do not change very often. However, there are two sources of possible change in the genetic makeup of bacteria:

- mutations (as seen in Section 3.1)
- plasmid transfer.

Plasmid transfer involves one bacterium donating genetic information to another in a ring of nucleotides called a plasmid. Both the donating and receiving cells open their cell walls so that the genetic material can pass from the donor to the receiver.

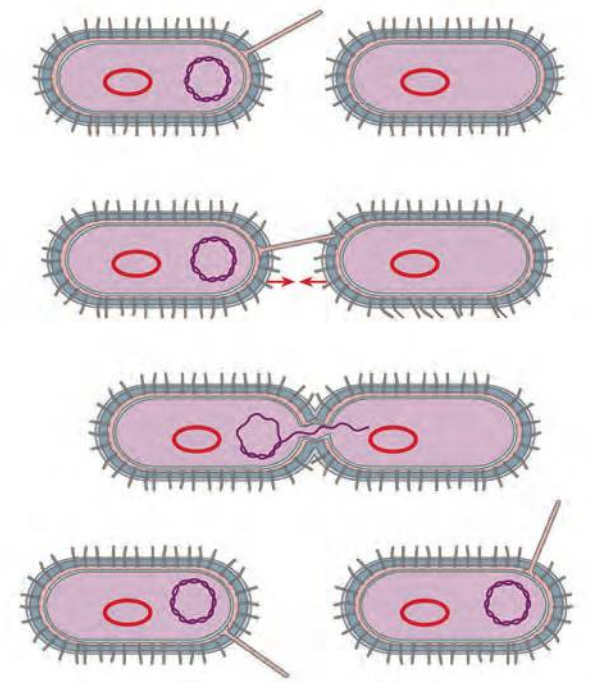


Figure 5.10 The bacterium on the left is passing genetic information to the bacterium on the right in a process called plasmid transfer.

The development of antibiotic-resistant bacteria has happened in several cases. New strains of syphilis, for example, have adapted to antibiotics and show multiple resistance. Some strains of tuberculosis are resistant to as many as nine different antibiotics. There is no cure for people who get sick from such super-resistant germs, and they must rely on their immune system to save them.

Finding new antibiotics would only be a temporary solution, and pharmaceutical companies cannot find new medications fast enough to treat these super-resistant germs. As a result, the best way to curb their expansion is to make sure that doctors minimize the use of antibiotics and that patients realize that antibiotics are not always the best solution to a health problem.

Notice how the two examples above are good illustrations of how we can use a scientific theory to explain observed phenomena. As stated at the beginning of the section on evolution, once the theory of natural selection is understood, it allows us to understand a variety of natural phenomena.

Antibiotic-resistant pathogens such as MRSA are causing hospitals and clinics all over the world to rethink their standards of hygiene. MRSA stands for methicillin-resistant *Staphylococcus aureus*. Health officials are concerned that, without internationally coordinated efforts, these super bugs could be spread from one country to another as patients get transferred across borders for treatment. What kinds of international regulations exist concerning antibiotic use, quarantine, and other such practices, that either encourage or limit the spread of resistant bacteria?

NATURE OF SCIENCE

A *Staphylococcus* bacterium discovered in a hospital is suspected of being resistant to a certain number of antibiotics. To test this hypothesis, the bacterium is introduced into a Petri dish along with small disks of paper that are soaked in different types of antibiotic. In an experiment like this, when the colonies of bacteria grow close to the disks, they show resistance to the antibiotic, whereas when wide, clear circles of inhibited bacterial growth are present, they show that the antibiotic is stopping the bacteria the way it should. Can you interpret the results of the experiment shown in the photo?

Testing for antibiotic resistance.

In the photo, the four disks of different antibiotics nearest the technician's hand show rings of growth inhibition, suggesting an effective control of the colony of bacteria by the medications. However, the two disks at the top furthest away from the hand (top centre and top left) have allowed the bacterial colony to grow dangerously close. This suggests that this strain of *Staphylococcus* is resistant to those two antibiotics and cannot be stopped by them. Doctors use such tests to help decide which medications to prescribe. In this case, they should prescribe the antibiotics that the bacteria do not show a resistance to, preferably the three at the bottom of the image.

This resistant bacterium is part of a growing number of super bugs, among which we find MRSA. They have evolved because of the way humans use antibiotics.

TOK

In some countries, there is a very intense debate about whether the concept of evolution should be taught in schools. To support the critics of evolution, there are thousands of websites and publications that carefully try to dismantle and disprove the arguments of evolutionary biologists. What criteria are used to determine whether these criticisms are valid or not? What kind of evidence would be necessary to refute Darwin's theory?

Evolution by natural selection is a multi-step process. Some steps involve chance, such as variation in a population, or certain aspects of sexual reproduction, such as which gametes participate in fertilization and which do not. However, the presence of a particular characteristic in a population is not purely up to chance. It's not just lucky, for example, that falcons have excellent vision or that dolphins are capable of echolocation. It's not by pure happenstance that flowers have adaptations perfectly suited to their insect pollinators, or that certain bacteria become resistant to the antibiotics we try to fight them with. Natural selection favours useful adaptations and selects against harmful ones in a way that is not based on luck and chance, but on fitness. Heritable changes are passed on from generation to generation, and accumulate over time so that each population either fits its environment, adapts accordingly, or dies out.

Design an experiment simulating natural selection

Safety alerts: When choosing objects used for simulating mouthparts or food, avoid objects that are too sharp, such as certain types of tweezers or thumb tacks. Also, if several competing organisms are trying to get food from the same food source, such as a tray or plate, you should not peck at your competition with your mouthparts.

In order to simulate natural selection between organisms obtaining food, design a lab in which some form of pinchers or clips are used as 'mouthparts' and a variety of small objects are used as 'food'. Some form of 'stomach' needs to be established, such as a Petri dish placed at a particular distance from the food source.

- Examples for mouthparts: tweezers, clothespins, wooden tongs, or even chopsticks.
- Examples for food: dry chickpeas or kidney beans, dry grains of rice, marbles, paper clips, or coins. To make it more challenging, calorie values could be given so that the most difficult food to pick up is worth the most calories.

The investigation should involve participants simulating organisms using their mouthparts (the tweezers, for example) to fill their stomachs with food. Those who attain a minimum requirement of food are allowed to continue to the next round; those who do not are eliminated by natural selection. In effect, the simulated organism dies of hunger.

The designed investigation must show a certain amount of variation of mouthparts within the population of feeding organisms. The investigation must also limit the time and the resources available. Natural selection should be demonstrated by determining a minimum amount of food collected in the organism's stomach within the time limit. Rules must be established to avoid cheating such as holding the stomach under the desk and pushing food into it.

Just as with any designed investigation, be sure to start with the aim, research question, and three types of variables, before establishing the step-by-step method. See the Internal assessment chapter in the eBook for help with variables. Some trial runs will probably be necessary to refine your method.

Exercises

5 Besides mutation, list two factors that are responsible for increasing variation in a population.

6 Distinguish between artificial selection and natural selection.

7 Ground-nesting birds such as grouse lay their eggs in a nest made on the ground. The eggs of this species are generally speckled dark brown. If a mutation occurred causing the eggs to be brightly coloured, how would the change in colour affect their chances of survival?

8 Explain how a population of insects could develop resistance to the insecticides sprayed on them.

To learn more about evolution, go to the hotlinks site, search for the title or ISBN, and click on Chapter 5: Section 5.2.

5.3 Classification of biodiversity

Understandings:

- The binomial system of names for species is universal among biologists and has been agreed and developed at a series of congresses.
- When species are discovered they are given scientific names using the binomial system.
- Taxonomists classify species using a hierarchy of taxa.
- All organisms are classified into three domains.
- The principal taxa for classifying eukaryotes are kingdom, phylum, class, order, family, genus, and species.
- In a natural classification, the genus and accompanying higher taxa consist of all the species that have evolved from one common ancestral species.
- Taxonomists sometimes reclassify groups of species when new evidence shows that a previous taxon contains species that have evolved from different ancestral species.
- Natural classifications help in identification of species and allow the prediction of characteristics shared by species within a group.

NATURE OF SCIENCE

Cooperation and collaboration between groups of scientists: scientists use the binomial system to identify a species rather than the many different local names.

Applications and skills:

- Application: Classification of one plant and one animal species from domain to species level.
- Application: Recognition features of Bryophyta, Filicinophyta, Coniferophyta, and Angiospermophyta.
- Application: Recognition features of Porifera, Cnidaria, Platyhelmintha, Annelida, Mollusca, Arthropoda, and Chordata.
- Application: Recognition of features of birds, mammals, amphibians, reptiles, and fish.
- Skill: Construction of dichotomous keys for use in identifying specimens.

Guidance

- *Archaea, Eubacteria, and Eukaryote should be used for the three domains.*
- *Members of these domains should be referred to as archaeans, bacteria and eukaryotes.*
- *Students should know which plant phyla have vascular tissue, but other internal details are not required.*
- *Recognition features expected for the selected animal phyla are those that are most useful in distinguishing the groups from each other, and full descriptions of the characteristics of each phylum are not needed.*
- *Viruses are not classified as living organisms.*

The binomial system of names for species

You have a name that you were given when you were born, but you also have a scientific name based on your species: *Homo sapiens*. This system of naming organisms using two names is called binomial nomenclature. 'Bi' means two, 'nomial' means name and 'nomenclature' refers to a system used to name things.

Myrmecophaga tridactyla is a name that literally means 'eater of ants' plus 'with three fingers'. In case you have not guessed, it refers to an anteater, and this one happens to be the giant anteater of Central and South America. In fact, the animal really has five fingers, but they are hard to see because the animal walks on its front knuckles.

Figure 5.11 The giant anteater, *Myrmecophaga tridactyla*.

The first name in the binomial nomenclature system is always capitalized and it refers to the genus; the second name always begins with a small letter and refers to the species. Both are always written in italics when typed, or underlined when written by hand. Most words used in binomial nomenclature are Latin or Greek in origin. For example, *Lepus arcticus* is the scientific name for the Arctic hare; both terms come from Latin. This is why the term Latin name is often used, although this is an oversimplification because other languages are also involved.

This system of naming organisms was consolidated and popularized by the dynamic Swedish naturalist Carolus (Carl) Linnaeus. In his book *Systema Naturae* (*The Natural World*, 1735), he listed and explained the binomial system of nomenclature for species that had been brought to him from all over the world. Although he was not the first to use the idea of genus (plural genera), he popularized its use along with the species name in a consistent way.

Today, there are hundreds of specialists who, like Linnaeus, describe and name new species. When it comes to classifying animals, for example, every 4 years the International Congress of Zoology takes place in a different city; it is an event during which animal experts from all over the world share and discuss their findings about animal behaviour, genetics, and classification. The dates and locations of the 19th–22nd congresses are:

- 2004 Beijing, China (XIX)
- 2008 Paris, France (XX)
- 2012 Haifa, Israel (XXI)
- 2016 Japan (XXII).

Zoologists started these conferences in Paris in 1889, on the occasion of the World Fair that year, the one that inaugurated the Eiffel Tower. Although many things are discussed as such congresses, one of the topics that comes up is the binomial nomenclature system. Decisions need to be made about new organisms that have been recently discovered or old organisms that might need reclassifying because of new evidence about their ancestry.

There are three main objectives to using binomial nomenclature and its associated rules: (1) to be sure that each organism has a unique name that cannot be confused with another organism; (2) so that that the names can be universally understood, no matter what nationality or culture is using the name; and (3) so that there is some stability in the system by not allowing people to change the names of organisms without valid reasons.

One result of discussions between many zoologists has been the International Code of Zoological Nomenclature (ICZN), which makes the rules about how to classify and name animals. There are also rules about how to use the names and properly cite them in research papers. In cases where two different animal species have been given the same name, there is a rule that the oldest valid publication of the name should be used. This is referred to as the principle of priority and is taken very seriously. This principle is applied when the same species is accidentally named twice by two different experts with two different names; again the first one gets priority.

In the days when there were fewer rules, some scientists named unsightly or offensively smelling organisms after people they considered to be their enemies. This is no longer allowed.

In addition to these zoological congresses to discuss animals, there are international congresses for many forms of life, including algae, fungi, plants, and bacteria, and each one has their own code for nomenclature. In this way, when a biologist discovers a new organism, he or she has detailed guidance from such codes about where to place the organism in the tree of life, a metaphor used to denote the branches leading back to a common ancestor.

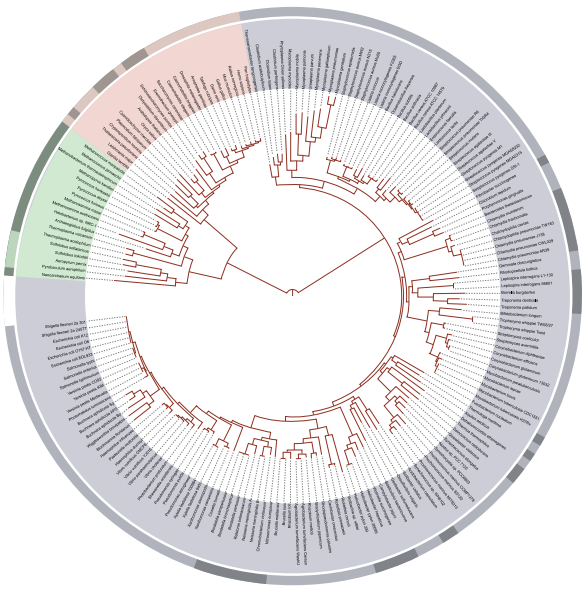


Figure 5.12 A diagram from the interactive Tree of Life online tool. Notice how, unlike other 'tree' diagrams, there is no summit on this circular diagram. All organisms alive today have evolved for the same number of years: we are all survivors. Species names are given around the outside of the circle. Find out more about this by going to the hotlinks site and clicking on Chapter 5: Section 5.3.

International cooperation and communication are key concepts in science. It is important that scientists are able to share their ideas, discuss developments, and make decisions together about how to communicate better and share knowledge. The continuing development of the binomial nomenclature system is an example of scientists recognizing and overcoming the confusion that would occur if each biologist used the local names of species in his or her own language. Although the original purpose of the internet was to serve military needs, the first major non-military group of individuals to see the usefulness of such a system was scientists.

Naming new species

Humans like to see similarities and differences in the objects that surround them: hot or cold, delicious or foul-tasting, dangerous or safe, and so on. In the early days of classification, all known organisms were classified into only two kingdoms: plants and animals.

As the centuries went by, and as the study of biology became more systematic, tens of thousands of new species were discovered in forests, deserts, and oceans, some of which showed characteristics of both plants and animals, and some of which were not like either plants or animals. For example, mushrooms grow on the forest floor the way plants do, and yet they do not have leaves or roots and they do not photosynthesize: they get their energy from digesting dead organic matter. So mushrooms cannot be classified as plants, because they are not autotrophs, but they are certainly not animal-like either, one reason being that they have cell walls made of chitin.

With the invention of the microscope in the mid 1600s, many new creatures were discovered that were nothing like plants or animals. In effect, the microscope revealed that there is an entire world of invisible organisms living throughout the biosphere.

If a botanist finds a new species of orchid, for example, he or she would have to describe the plant, describe the location it was found in, name it using the proper rules of binomial nomenclature as set out by the International Code of Botanical Nomenclature (ICBN), and publish the findings in a publically accessible publication. In addition, it is important to put a sample specimen in a public location where other botanists can examine it. Such an example specimen is called a holotype. One of the rules of nomenclature is that a scientific name is not considered valid if a specimen is not available for verification. In some circumstances, a precise illustration is acceptable, but it is always better to make a holotype available. Proposing a name for mythical creatures no one has ever captured, for example, is not accepted.

On the other hand, it is perfectly acceptable to name a well-described organism that no longer exists, such as an extinct dinosaur. Usually the holotypes of fossilized species are kept in museums, but simply finding a fossil, labelling it and putting it on display in a museum does not count as officially naming it. Again, the name would have to be published along with a description in a reputable scientific publication.

This fossil skull was discovered by Mary Leakey in 1959 at Olduvai Gorge, Tanzania. It is the holotype for the extinct hominid species *Paranthropus boisei* and the skull is now at the Natural History Museum in London.

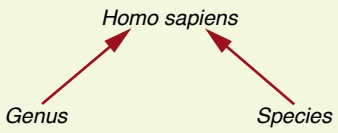
Examples of binomial nomenclature

Sometimes scientific names for organisms are relatively easy to decipher because they contain their common names:

- *Amoeba amazonas*
- *Equus zebra*
- *Gekko gecko* (this lizard gets its name from the sounds it makes).
- *Gorilla gorilla*
- *Paramecium caudatum* (caudate means having a tail).

Sometimes, it is more difficult to guess their common name:

- *Apis mellifera* (honeybee, although you might have guessed this if you know that beekeeping is also called apiculture)
- *Aptenodytes patagonicus* (king penguin, although you can probably guess where it lives from its species name)
- *Loxodonta cyclotis* (African forest elephant)
- *Malus domestica* (apple tree).



The rules about writing binomial nomenclature names are that:

- **the genus name is capitalized but the species name is not**
- **both are written in italics when typed, or underlined when handwritten**
- **in addition, after these two names, often the last name of the person who first published the name in a scientific journal is given (but not italicized), and the date when it was published, for example *Equus zebra* Linnaeus, 1758.**

Scientists naming organisms sometimes have a sense of humour. Here are a few examples.

- *Albunea groeningi* Boyko, 2002. This sea snail was named after the cartoonist who created 'The Simpsons': Matt Groening.
- *Agra schwarzeneggeri* Erwin, 2002. This Costa Rican ground beetle was named after Arnold Schwarzenegger because of the insect's large biceps.
- *Dracula vampira* Luer, 1978. This orchid in Ecuador got its name from the fact that the petals on the flower look like a bat's wings.
- *Spongiforma squarepantsii* Desjardin, Peay & T.D. Bruns, 2011. This orange-coloured mushroom from Borneo gets its name from the children's cartoon character SpongeBob SquarePants.

A hierarchy of taxa

The term taxa (singular taxon) refers to the categories that scientists have generated names for. You can think of taxa as being like folders for organizing your school papers. Just as you would not (or should not) file your history notes in your maths folder, so biologists do not put birds in the same category as mammals. Likewise, within your history folder, you might have subfolders for homework, notes, tests, and so on. Within the category of plants, biologists have smaller categories for flowering plants, conifers, spore-producing plants, etc. Thus a hierarchy of taxa is used to classify species into many subcategories that are found within larger categories. There are specific names for these categories.

What do we do with viruses? How do we classify them? Viruses contain genetic information and yet they cannot reproduce outside a host cell; they do not feed, grow, or metabolize in the way that living organisms do, so they are considered to be non-living. For taxonomists, viruses are not classified as living things: they do not fall anywhere in the three domains. As a result, they are treated separately, and virologists have their own classification system.

Three domains of life

At the top of the hierarchy are the three largest groupings for organisms, called domains. The names of these three domains are the Archaea domain, the Eubacteria domain and Eukaryote domain. All living organisms are classified into one of these three. Note that viruses are not in this list because they are not alive and do not necessarily share a common ancestry with each other, two major conditions necessary to fit into this classification system (Figure 5.20 in the next section shows how the three domains are related.).

Archaeans are single-celled organisms that are distinct from bacteria and are very ancient. Archaean species thrive today in diverse habitats, from extreme conditions such as hydrothermal vents and hot springs, to the guts of mammals. Some of the beautiful colours of hot springs in places such as Yellowstone National Park are because of the presence of archaeans. The types of archaeans that prefer extreme conditions are called extremophiles and include thermophiles (heat-loving), methanophiles (methane-loving), and halophiles (salt-loving).

Grand Prismatic Thermal Springs in Yellowstone National Park. The bright colours around the edge of the hot water are caused by microbial colonies that include archaeans.

Halocins are types of antibiotics made by halophile (salt-loving) archaeans. Just as penicillin was first discovered in a fungus, lots of pharmaceutical drugs come from naturally occurring compounds. Archaeans are currently being studied for the types of organic molecules they can produce, and some of them may hold the key to fighting diseases for which we do not yet have a cure.

Eubacteria is the domain in which we find the bacteria you are most familiar with: the kind that makes your yogurt taste good, the kind that helps your intestines work properly, and also the kind that might give you an infection.

Eukaryote is the domain in which we find all other life besides Archaea and bacteria, from the microscopic single-celled yeast that helps bread to rise, to enormous organisms such as sequoia trees and blue whales. A eukaryote is recognizable by its membrane-bound nucleus and membrane-bound organelles.

Seven principal taxa

In order to classify the hundreds of thousands of different types of organisms on Earth, scientists have agreed to use a seven-level hierarchy of taxa. Each of the three domains is subdivided into these seven taxa:

- kingdom
- phylum
- class
- order
- family
- genus
- species.

The taxa that are higher up this list contain the most numbers of organisms, and the taxa at the bottom of the list contain the least number. For example, although there are hundreds of thousands of named animals in the Eukaryote kingdom (most of which are insects), there is only a single known species of humans on Earth today: *Homo sapiens*. So the higher taxa have very general characteristics encompassing many types of organisms, and the lower taxa have increasingly specific characteristics; the hierarchy narrows the categories down into smaller and smaller numbers of subcategories.

Table 5.2 shows two examples of the full identification of two species according to the seven taxa we have just named.

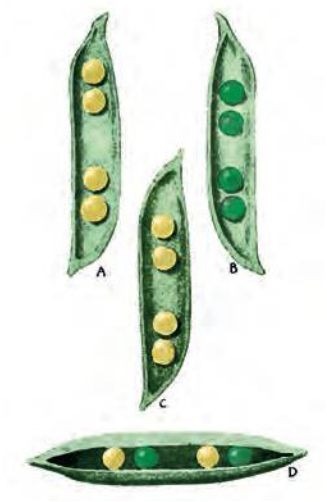


Figure 5.13 The garden pea, *Pisum sativum*, is the plant Gregor Mendel studied.

Table 5.2 The classification of two species.

Taxa	Human	Garden pea
Kingdom	Animalia	Plantae
Phylum	Chordata	Angiospermophyta
Class	Mammalia	Dicotyledoneae
Order	Primate	Rosales
Family	Hominidae	Papilionaceae
Genus	*Homo*	*Pisum*
Species	*sapiens*	*sativum*

To help remember the order of the taxa, a mnemonic (memory trick) is helpful. Make a sentence using the first letters of each level, such as 'King Philip Came Over For Good Soup'. The human brain is very poorly adapted for remembering lists of words but very highly adapted for remembering stories. Transforming lists into stories is a good example of a mnemonic.

Other classifications

The system of kingdoms and taxa is used for identifying and naming organisms, but there are countless other ways to classify organisms. Here are some examples:

- by feeding habits: carnivore/herbivore
- by habitat: land dwelling/aquatic
- by daily activity: nocturnal/diurnal
- by risk: harmless/venomous
- by anatomy: vertebrates/invertebrates

No single classification system is the 'right' way. Think of all the ways that the students in a class could be put into different groups: by eye colour, by shoe size, by birth date, by academic results, by favourite musical group, by alphabetical order, by length of fingernails, by what they had for breakfast! What is important for a system of classification is that it is clear, consistent, logical, easily implemented, and that there is a general consensus to apply it.

CHALLENGE YOURSELF

2 Look up the following things to find out what their scientific names are:
- your favourite animal
- your favourite food
- your favourite flower, tree, or house plant.

A common ancestral species

In biology, one of the objectives of classification is to represent how living (and extinct) organisms are connected. This means we are interested in natural classification, classifying organisms by their descent from a common ancestor. In Linnaeus' time, a century before Darwin and Mendel's work, the existence and function of DNA was not known, so classifications were based on observable characteristics. Today, it is preferable to use ancestry and genetics to classify organisms. The best way to establish a natural classification is to base it on DNA sequences. When the sequences are not available, the next best way is to look at derived characteristics, such as whether or not an organism can produce milk. There will be more about derived characteristics in Section 5.4.

When genetic similarities are found, a genus can be established in which all similar species are placed. The members of this genus will have all evolved from a common ancestor, and this will be evident in the similarities between their gene sequences.

Without a universal classification system, each language, culture, or region may have a different name for an organism. For example, the pill bug and woodlouse sound like two different organisms but they are, in fact, the same one: *Armadillidium vulgare*. The common names do not reveal anything about a species' evolutionary links, but its scientific name does.

You may have come across this kind of invertebrate under rotting logs.

Reclassification

As noted before, Linnaean classification was limited to observable characteristics, and in Linnaeus's time little effort was made to classify organisms by their ancestry because nothing was known about the genetic connections between species. The consequence of this is that sometimes organisms were put in the same genus even though they are not in fact closely related to each other. With a better understanding of cell structure and metabolism, as well as the new techniques of gene sequencing developed over the past few decades, we now know that some organisms that were put into the same categories in the 1700s should not be together in the same genus or even the same order.

Today, many species have been reclassified. A good example is a group of flowers called asters that were all formerly in a genus called *Aster* that comprised hundreds of species distributed widely across geographical and temperature ranges at various altitudes in Europe, Asia, and the Americas. Many species of these plants are cultivated in gardens for their decorative flowers (an example is shown in the photo on page 247). In recent decades, taxonomists have split this group into species that can trace their

ancestry to the Old World (Europe and Asia) and species that can trace their ancestry to the New World (North, Central and South America).

Looking at the ancestry of the asters, revealed in part by the structure of the single-seeded fruit they make called an achene, it was decided that there was a significant enough difference between the species on the two sides of the Atlantic Ocean that reclassification was necessary. The new classification is a better reflection of which ones are more closely related to each other. Of the genera that were put into the New World group, one example is the blue wood aster, which has now been placed in the genus *Symphyotrichum*. Table 5.3 shows what the reclassification has done to the blue wood aster's scientific name.

Old classification	New classification
Aster cordifolius	*Symphyotrichum cordifolium*

Table 5.3 The classification of the blue wood aster

Blue wood aster.

One of the challenges to renaming organisms is that books and scientific journals, as well as gardening guides and museum herbarium collections, often still have the old scientific names. This means that, before using a scientific name, it is best to check that the name respects any recent reclassifications. Fortunately, with online databases and user-generated content in web-based encyclopaedias, names can be updated and notes can be left about the previous name, so that specialists doing research can usually find a species whether or not a new or an old name has been used. One such online

How are taxonomists classified? Answer: into lumpers and splitters. In taxonomy, there are two opposing philosophies concerning what to do when an organism does not fit well into existing categories: (1) broaden the definition of an existing category to include the new organism; or (2) invent a new category or subcategory. Specialists who take the first approach are referred to as lumpers, and those who take the second approach are referred to as splitters. As you can imagine, there can be lengthy discussions between the two groups. Generally speaking, lumpers focus on the similarities between organisms, while splitters focus on the differences between organisms.

database is the Integrated Taxonomic Information System (ITIS), which you can find in the hotlinks at the end of this section.

Another challenge is that, just because a group of taxonomists decides to make a change, it does not mean that everyone will agree with that change. In addition to resistance to breaking with tradition, or the insistence of some taxonomists to maintain stability in a name no matter what, there may be some scientists who disagree with the way new groups have been determined. Just because a committee of taxonomists insists that a certain difference in cell structure is a significant enough reason to change a classification, does not mean that everyone will embrace the decision. This is one of the reasons why, long after a decision has been made, it is still possible to see an older name in field guides, databases, scientific journals, and museum labels.

Natural classification

Natural classification uses ancestry to group organisms together, whereas artificial classifications use arbitrary characteristics, such as whether or not a plant or animal tastes good, or is useful to the textile industry, or whose name begins with the letter 'c'. You may laugh, but early classification systems were often based on listing the species by alphabetical order, the way a dictionary lists words. The reasons for putting living organisms into groups according to a natural classification rather than an artificial one are numerous, and include:

- trying to make sense of the biosphere
- showing evolutionary links
- predicting characteristics shared by members of a group.

If you find a type of sea creature that you have never seen before, you should be able to find an identification key that was made by the experts who classified it. If you do a comprehensive search in the published literature of organisms that have already been identified and do not find a name for the organism, it is possible that you have discovered a new species. To put it into its appropriate category, you would find currently existing taxa that contain similar organisms. You would determine whether it had a backbone or not, if it had stinging cells or not, and so on, until you reached a family or genus that it fit into. Once you find that genus, you can look at the list of characteristics of the species in that genus and make predictions about your new species. You might be able to predict what it eats, how long it lives, whether or not it produces certain enzymes, or even certain characteristics about its cell structure or biochemistry.

In the other direction, if biologists look at characteristics common to all life forms, such as the basic information in DNA about fundamental processes such as cellular respiration and cell division, they can deduce what the common ancestor to all life was like. This organism, sometimes named LUCA for last universal common ancestor, or LUA for last universal ancestor, lived over 3.5 billion years ago and parts of its DNA code can be worked out by retracing and examining the ancestries of various forms of life.

Below, you will see some of the characteristics that scientists look for when classifying organisms. We will look at plants and animals, but be aware that there are other kingdoms not mentioned here.

Examples of plant phyla

Of the several phyla of plants, four represent many of the types of plants you are probably most familiar with.

- Bryophyta: the bryophyte phylum includes plants of very short stature, such as mosses.
- Filicinophyta: this phylum includes ferns and horsetails, among others.
- Coniferophyta: the conifer phylum includes cedar, juniper, fir, and pine trees, among others.
- Angiospermophyta: the angiosperm phylum includes all plants that make flowers and have seeds surrounded by a fruit.

Let's examine each of these phyla more closely.

Bryophyta

Bryophytes, such as the liverwort shown below, are referred to as non-vascular plants because they do not have true vascular transport tissue inside them, such as xylem tissue (which transports water and minerals up from the roots) or phloem tissue (which transports water and nutrients from the leaves towards the stem and roots).

Filicinophyta

Members of the Filicinophyta, on the other hand, are vascular plants, as are the other two phyla described in this section. Ferns are recognizable by the absence of flowers and by their triangular fronds made up of many smaller long thin leaves.

Coniferophyta

Conifers can be recognized by the fact that all of them produce woody stems and their leaves are in the form of needles or scales.

A liverwort is an example of a bryophyte.

Trees that produce seed cones and have needle-like leaves are conifers.

This mass growing on the bark of a tree branch is also a bryophyte.

Examples from different plant phyla.

The chances are that you have eaten an angiosperm today: wheat, corn, apples, and oranges are all examples of angiosperm seeds and their coverings.

Angiospermophyta

The most obvious vegetative characteristic that allows angiosperms (i.e. members of the Angiospermophyta) to be identified quickly are their flowers and fruit. If the fruit has any seeds inside, the plant is an angiosperm.

The mosses, liverworts, and hornworts that make up the bryophytes do not produce flowers or seeds. Instead, they produce spores, which are microscopic reproductive structures. Bryophyte spores are transported by rainwater and ground humidity, which is one of the reasons why they are found most abundantly in damp habitats such as a forest floor. The same is true for the plants that are filicinophytes.

In contrast, all species of conifer use wind to help them reproduce by pollination. Most species of conifer produce seed cones with seed scales.

Although angiosperms also produce seeds, they do not produce cones and they are not always pollinated by wind. Many flowering plants rely on birds, insects, and sometimes mammals to transport their pollen from one flower to the next.

The sexual reproductive organs of angiosperms are their flowers. The fruit, which is the enlarged ovary of the plant, holds the seeds.

Examples of animal phyla

Of all the phyla of animals, we will consider seven here. Some of these you may be familiar with, but others you probably do not know much about. Only one of the categories of animals in these seven phyla has a backbone or vertebral column; they are called vertebrates. The other six categories are all invertebrates: they do not have a backbone.

- Porifera: this phylum consists of the sponges.
- Cnidaria: this phylum includes sea jellies (jellyfish) and coral polyps, among others.
- Platyhelminthes: this phylum is made up of flatworms.
- Annelida: this phylum is made up of segmented worms.
- Mollusca: this phylum contains snails, clams, and octopuses, among others.
- Arthropoda: this phylum includes insects, spiders, and crustaceans, among others.
- Chordata: these are the vertebrates, the animals that have a backbone.

A yellow tube sponge, one of the members of the phylum Porifera.

Porifera

Sponges are marine animals that are sessile (i.e. they are stuck in place). They do not have mouths or digestive tracts. Rather, they feed by pumping water through their tissues to filter out food. They have no muscle or nerve tissue and no distinct internal organs.

Cnidaria

Cnidarians are a diverse group, including corals, sea anemones, jellyfish (sea jellies), hydra, and floating colonies such as the Portuguese man-of-war. This diversity makes it difficult to give an overall description of common characteristics. However, one feature that unites cnidarians is that they all have stinging cells called nematocysts.

Some of these organisms are sessile, others are free-swimming, and some can be both depending on the period of their life cycle. To digest the food they catch in their tentacles, they have a gastric pouch with only one opening. Some of the free-floating species are carried by the current, but others are agile swimmers.

Platyhelminthes

Flatworms have only one body cavity: a gut with one opening for food to enter and waste to exit. They have no heart and no lungs. One of the most famous, or infamous, members of this phylum is the parasitic tapeworm that can infest the intestines of mammals, including humans. The reason for a flatworm's flat shape is that all the cells need to be close to the surface to be able to exchange gases by diffusion. Their bodies are not segmented (divided up into sections).

The common earthworm is an annelid.

Annelida

Annelids are the segmented worms, such as earthworms, leeches, and worms called polychaetes. Here, the word segmented refers to the fact that their bodies are divided up into sections separated by rings. Annelids have bristles on their bodies, although these are not always easily visible. Like the next two phyla, annelids have a gastric tract with a mouth at one end and an opening at the other end where wastes are released.

Mollusca

Most molluscs are aquatic, and include snails, clams, and octopuses. Many produce a shell reinforced with calcium. Like annelids, they have a one-way digestive system with both a mouth and an anus. But, unlike annelids, their bodies are not segmented.

Spiders are arthropods..

Arthropoda

Arthropods have a hard exoskeleton made of chitin, segmented bodies, and limbs that can bend because they are jointed. Although the limbs are often used for walking, some are adapted for swimming, and others can form mouthparts.

Arthropods include insects, spiders, and scorpions, as well as crustaceans such as crabs and shrimps. They are true champions of diversity and adaptation because they have conquered most habitats worldwide; there are more than a million species of arthropod. They vary in size from the most minute mites, just over 100 µm long, to the Japanese giant spider crab, which is 4 m in length.

Chordata

The chordates are organisms that have a notochord at some point in their development. A notochord is a line of cartilage going down the back that provides support to the animal. It is always present at one stage in the development of a chordate organism, but can be absent from other stages. The vast majority of animals in this phylum have a bony backbone, such as birds, mammals, amphibians, reptiles, and fish, although some fish such as sharks have a cartilaginous spine instead of one made of bone. Unlike the six previous examples, these organisms are all called vertebrates. There are some exceptions to the generalization that all chordates have a backbone: sea squirts do not, for example, but are still classified in this phylum because they do develop a notochord.

When we say the word 'animal' to a child, he or she will probably think of animals with backbones, perhaps because many children's books feature vertebrates as the main characters. To a biologist, vertebrates are relatively rare; invertebrates, such as insects, are much more common on Earth.

The vertebrates

We will now explore the characteristics used to classify vertebrate organisms into the following five classes:

- fish
- amphibians
- reptiles
- birds
- mammals.

Fish

From goldfish to sharks, fish are a class of very diverse aquatic organisms that possess gills to absorb oxygen, and have skulls made of bone or cartilage. Great white sharks are well known for their jaws and teeth, and the vast majority of fish have these features, although they are not always visible. A small number of fish, such as lampreys, are jawless and use their mouths as suckers to stick onto a surface. Although fish can have limbs in the form of fins, none of the limbs have digits (fingers). Some marine mammals, such as whales, orcas, and dolphins, might resemble fish but are not, one reason being they have articulated bony fingers inside their fins.

A tadpole is the larval stage of an amphibian such as this frog. In this photo, the young frog is almost ready to leave the water because its four limbs have developed.

Amphibians

Amphibians include organisms such as frogs and salamanders; they start their lives in water. Their larval forms usually have gills to breathe underwater, but their adult forms develop lungs for breathing air. Most amphibians can also absorb oxygen through their skin. Most have four legs when they are adults, but there is a legless group called caecilians that resemble large worms or small snakes. They eat a wide variety of food, which they can chew with teeth. They might seem similar to reptiles, but their eggs do not have a membrane around the embryo. Like reptiles, however, amphibians cannot control their body temperature; they are called ectothermic (or, more informally, cold-blooded) and need to bask in the sunshine to warm up, and seek shade or water to cool off.

Reptiles

Organisms such as snakes, lizards, turtles, and alligators are classified as reptiles in part because they produce amniote eggs. Amniote eggs are characterized by having a membrane around the developing embryo to protect it, which is seen not only in reptiles with soft or hard-shelled eggs but also in birds and mammals. What sets reptiles apart from other animals is that they have scales on their body instead of feathers or fur. Like amphibians, reptiles are ectothermic; they cannot regulate their body temperature.

This marine iguana needs to bask in the sun to warm up after a cold swim in the ocean. Notice the scales covering the body, and notice the pentadactyl forelimb.

Birds

All living species of birds are bipedal (have two legs) and possess wings, most of which are adapted for flight. All birds have feathers and lay eggs with hardened shells. Bird skeletons are often very lightweight, making them well-adapted for flight. Their low density is achieved by having hollow bones. Penguins are an example of a flightless bird, but their wings are well-adapted for swimming. Birds are also characterized by the fact that their jaws are in the form of beaks with no teeth, and they usually build nests for their young, albeit in a variety of places, such as in trees, on the ground, on cliff faces, and on urban structures. Their heartbeat and breathing rates are relatively fast because they have a high rate of metabolism.

Mammals

Mammals include animals such as foxes, hippopotamuses, squirrels, and camels, and can be recognized by the fact that they have hair on their bodies and the females produce milk in specialized glands to feed their young. There are nearly 5500 species of known mammals in the world, most of which have four limbs adapted for life on land. Some mammals, such as whales and dolphins, are adapted for life in the water, and others, such as bats, are adapted for flight. Mammals are capable of thermoregulation: they maintain their body temperature at a fixed level.

Using a dichotomous key

When biologists encounter a species they do not recognize, they use a dichotomous key to establish which taxa it belongs to. If you have ever played a guessing game in which the rule is that you can only ask 'yes' or 'no' questions, then you already know how a dichotomous key works. Here are the basic principles.

1. Look at the first section of the key, which has a pair of sentences, (a) and (b), describing characteristics.
2. Next, look at the organism to see if the particular characteristic described in the first line (a) is present in the organism.
3. If the answer is yes, then go to the end of its line and find the number of the next pair of statements to look at, follow the number given and continue until the end. If the end of the line contains a name, it is the taxon for the organism.
4. If the answer is no, then go to the second statement just below it (b) and that one should be true, so go to the end of its line and find the number of the next pair of statements to look at. Follow the number given and continue until the end.

Keep going until you get to a name instead of a number: if you have answered each question correctly, that will be the name of the taxon your organism belongs to. Try identifying the organisms shown opposite using the key in the following example.

Worked example

Here is an example of a key for identifying the animal taxa listed in this chapter.

1	(a) No differentiated tissues, no symmetry or identifiable organs	Porifera
	(b) Presence of differentiated tissues and organs	2
2	(a) Stinging cells present, can show radial symmetry	Cnidaria
	(b) No stinging cells	3
3	(a) Has two-way digestive tract and bilateral symmetry	Platyhelminthes
	(b) Has a one-way digestive tract (mouth and anus)	4
4	(a) Does not possess a notochord at any time	5
	(b) Possesses a notochord at some stage	7
5	(a) Has an exoskeleton made of chitin	Arthropoda
	(b) Does not have an exoskeleton made of chitin	6
6	(a) Has a segmented body	Annelida
	(b) Makes a shell reinforced with calcium	Mollusca
7	(a) Four limbs present, with articulated digits	8
	(b) Limbs present, but they do not have digits	Fish

8 (a) Does not produce an amnion..... Amphibians
(b) Can produce an amnion..... 9
9 (a) Presence of hair on the body, can make milk to feed young ..... Mammal
(b) Absence of hair, cannot make milk 10
10 (a) Body covered with feathers..... Bird
(b) Body covered with scales ..... Reptile

Use the key to find out which taxon each organism pictured below is in. Show how you did your work by writing the numbers and letters you followed.

1

2

3

4

Solutions

1 1b →2b →3b →4a →5a = Arthropoda
2 1b →2a = Cnidaria
3 1b →2b →3b →4b →7a →8b →9b →10b = Reptile
4 1b →2b →3b →4a →5b →6b = Mollusca

Exercises

9 List the three classification domains. Determine which domain each of the following organisms belongs to.

(a) A single-celled organism that prefers very salty water.
(b) Algae (hint: they have a nucleus).
(c) Spider.
(d) *Escherichia coli*.

10 Suggest one reason why viruses do not fit into the three-domain system.

11 Make a table with four columns headed Bryophyta, Filicinophyta, Coniferophyta, and Angiospermophyta. Make two rows labelled 'Physical characteristics' and 'Named examples'. Complete the eight empty cells of the table.

12 In the seven-taxa system, state the order that you belong to.

13 Using 10 different objects found in your school bag, design a dichotomous key.

CHALLENGE YOURSELF

3 Construct your own dichotomous key for use in identifying specimens. Because the example shown is for animal taxa in this chapter, try one for the plant taxa described in this chapter.

Campers and hikers can use a dichotomous key in a field guide to be sure that any mushrooms or plants they find are edible and not poisonous. They can also use a key to determine whether or not certain plants are endangered or protected species.

TOK

In his classification of organisms, Linnaeus used physical characteristics and social behaviour to establish four groups of humans. Reading such descriptions today is shocking because, by modern standards, they have a racist nature. To what extent is it necessary to consider the social context of scientific work when evaluating ethical questions about research?

To learn more about taxonomy and classification, go to the hotlinks site, search for the title or ISBN, and click on Chapter 5: Section 5.3.

NATURE OF SCIENCE

Falsification of theories with one theory being superseded by another: plant families have been reclassified as a result of evidence from cladistics.

5.4 Cladistics

Understandings:

- A clade is a group of organisms that have evolved from a common ancestor.
- Evidence for which species are part of a clade can be obtained from the base sequences of a gene or the corresponding amino acid sequence of a protein.
- Sequence differences accumulate gradually so there is a positive correlation between the number of differences between two species and the time since they diverged from a common ancestor.
- Traits can be analogous or homologous.
- Cladograms are tree diagrams that show the most probable sequence of divergence in clades.
- Evidence from cladistics has shown that classifications of some groups based on structure did not correspond with the evolutionary origins of a group or species.

Applications and skills:

- Applications: Cladograms including humans and other primates.
- Application: Reclassification of the figwort family using evidence from cladistics.
- Skill: Analysis of cladograms to deduce evolutionary relationships.

Characteristics used for classification

Table 5.4 shows some types of characteristics that botanists and zoologists might study in order to help them decide how to classify an organism.

Table 5.4 Types of characteristics used for classifying organisms

Characteristic	Example/reason
Morphology	The shape of a plant's seed coat or the shape of a bird's bill
Anatomy	The number of petals on a flower or the type of digestive system in an invertebrate
Cytology	The structure of cells or their function
Phytochemistry	Special organic compounds that only plants can make, often to protect themselves from attack by insects
Chromosome number	Two species with the same chromosome number are more likely to be closely related than those with differing numbers
Molecular differences	Proteins and DNA sequences differ between one species and another

Classifying organisms using molecular differences is called molecular systematics. As technology is improved and becomes more affordable, more and more specialists are using methods involving protein sequences and DNA.

Clades

Cladistics is a system of classification that groups taxa together according to the characteristics that have evolved most recently. In this system, the concept of common descent is crucial to deciding into which groups to classify organisms. Cladistics is, therefore, an example of natural classification. To decide how close a common ancestor is, researchers look at how many primitive and derived traits the organisms share.

Primitive traits (also called plesiomorphic traits) are characteristics that have the same structure and function (e.g. leaves, with vascular tissue to transport liquids around a plant) and that evolved early on in the history of the organisms being studied. Derived traits (also called apomorphic traits) are also characteristics that have the same structure and function but that have evolved more recently as modifications of a previous trait (e.g. flowers, which evolved more recently than leaves with vascular tissue, i.e. they are an adaption of vascular leaves). By systematically comparing such characteristics, quantitative results show which organisms have a more recent split in the evolutionary past and which have a more distant split.

When a group can be split into two parts, one having certain derived traits that the other does not have, the groups form two separate clades. A clade is a monophyletic group. This means it is a group composed of the most recent common ancestor of the group and all its descendants. Although a clade can sometimes have just one species, usually it is made up of multiple species.

Biochemical evidence of clades

Biochemical evidence, including DNA and protein structures, has brought new validity and confirmation to the idea of a common ancestor. For example, the fact that every known living organism on Earth uses DNA as its main source of genetic information is compelling evidence that all life on Earth has a common ancestor. As you saw in Section 3.5 on genetic engineering, any gene from any organism can be mixed and matched with DNA from other organisms to generate a certain protein. Other than conceding that we all have a common ancestor, it would be difficult to explain how else this is true.

In addition, all the proteins found in living organisms use the same 20 amino acids to form their polypeptide chains. Again, this has been confirmed by the introduction of foreign genes using genetic engineering to get an organism to synthesize a protein that it never synthesized before.

Amino acids can have two possible orientations: left-handed and right-handed, depending on the way their atoms are attached together. The overwhelming majority of living organisms on Earth use left-handed amino acids to build their proteins, and only a small number of organisms (notably some bacteria) can use right-handed amino acids. For those who support the idea of the biochemical evolution of life, the most logical explanation for such chemical similarities is that they imply a common ancestry for all life forms that use left-handed amino acids to build their proteins.

Variations and phylogeny

Phylogeny is the study of the evolutionary past of a species. Species that are the most similar are most likely to be closely related, whereas those that show a higher degree of differences are considered less likely to be closely related. By comparing the similarities in the polypeptide sequences of certain proteins in different groups of animals, it is possible to trace their common ancestry. This has been done with the blood protein haemoglobin, with a mitochondrial protein called cytochrome *c*, and with chlorophyll, to name just three proteins.

With advances in DNA sequencing, the study of nucleic acid sequences in an organism's DNA, as well as its mitochondrial DNA, has been effective in establishing biochemical phylogeny. Changes in the DNA sequences of genes from one generation

to the next are partly due to mutations, and the more differences there are between two species, the less closely related the species are.

Here is an imaginary example of a DNA sequence from four different organisms:

1 A A A A T T T T C C C C G G G G
2 A A A A T T T A C C C C G G G G
3 A A A A T T T A C C C G C G G G
4 A A C A T C T A C C A G C C T G

The differences have been highlighted in red. It should be clear that species 1 and 2 have the fewest differences between them, whereas species 1 and 4 have the most differences. As we have seen in Chapter 4, these differences can arise as a result of mutations. The second sequence shows only one difference with the first, but the fourth shows eight differences. The conclusion could be that species 1 and 2 are more closely related to each other than they are to species 3 or 4.

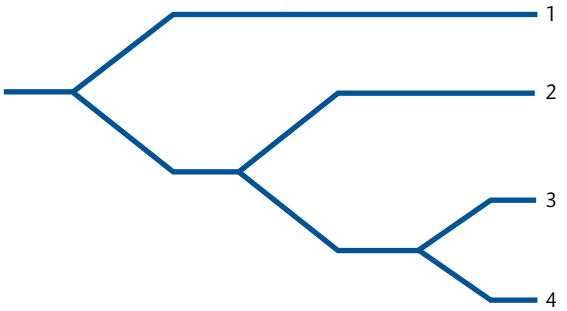


Figure 5.14 A representation of the relationships between four species

Figure 5.14 shows how these four imaginary species could be related:

Often, such work by biochemists confirms what palaeontologists have hypothesized about the ancestries of the fossils they have studied. When one branch of science confirms the work of another branch, the findings have more credibility. In other cases, the biochemical evidence can be contradictory, which encourages scientists to reconsider their initial ideas.

The evolutionary clock

Differences in polypeptide sequences accumulate steadily and gradually over time, as mutations occur from generation to generation in a species. Consequently, the changes can be used as a kind of clock to estimate how far back in time two related species split from a common ancestor.

By comparing homologous molecules from two related species, it is possible to count the number of places along the molecules where there are differences. If the molecule is mitochondrial DNA, for example, we count the number of base pairs that do not match. Mitochondrial DNA is particularly interesting to study because, unlike DNA found in the cell's nucleus, it is not shuffled and mixed during meiosis or fertilization: it is passed on directly from mother to child without modification. This is why we can be sure that any modifications in mitochondrial DNA are due solely to mutations.

Imagine comparing certain DNA sequences from three species, A, B, and C. Between the DNA samples from species A and species C there are 83 differences. Between species A and species B there are only 26 differences. From these data, we can conclude that species B is more closely related to species A than species C is. There has been more time for DNA mutations to occur since the split between A and C than since the split between A and B.

One technique that has been successful in measuring such differences is DNA hybridization. The idea is simple: take one strand of DNA from species A and a homologous strand from species B and fuse them together. Where the base pairs connect, there is a match; where they are repelled and do not connect, there is a difference in the DNA sequence and therefore there is no match (see Figure 5.15).

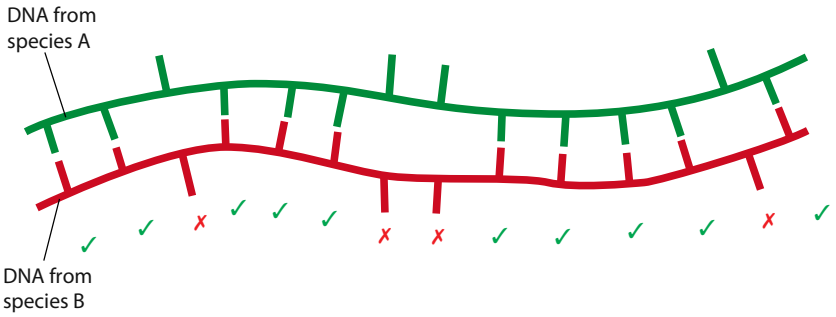


Figure 5.15 DNA hybridization between a strand of DNA from one species (in green) and another from a second species (in red). There are four places where a match does not occur.

We can take this further. If we see that 83 nucleotide differences is approximately three times more than 26 differences, we can hypothesize that the split between species A and species C happened about three times further back in the past than the split between the species A and B. This is the idea of using quantitative biochemical data as an evolutionary clock to estimate the time of the speciation events (see Figure 5.16).

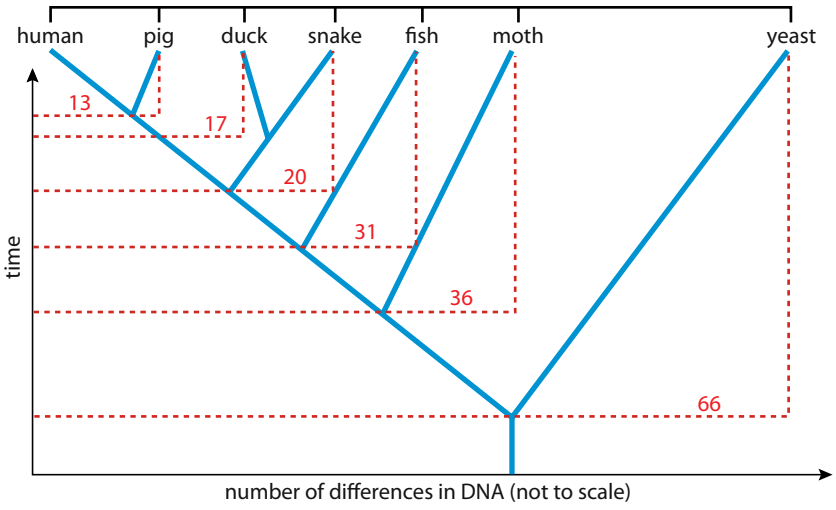


Figure 5.16 Biochemical differences (dotted red lines) can be used to see how far apart species are on a phylogenetic tree (in blue).

However, we need to be careful when using a word such as 'clock' in this context. Under no circumstances should we consider that the 'tick-tock' of the evolutionary clock, which is made up of mutations, is as constant as the ticking of a clock on the wall. Mutations can happen at varying rates. Consequently, all we have is an average, an estimation or a proportion, rather than an absolute time or date for speciation events. In an effort to double-check the timing of the evolutionary clock, biochemical data can be compared with morphological fossil evidence and radioisotope dating.

Experts in various fields of study use this idea of accumulated change over time. For example, linguists look at changes in words and uses of vocabulary to trace the evolution of a language throughout the course of history. Some language experts can deduce when pigs were domesticated in a particular country just by looking at the names for 'pig' in the various languages in and around that country. Experts who study chain letters sent by the post or by email are interested in the number of modifications to the original letter over time. By comparing hundreds of versions of the same message, they can analyse what has been added or changed to see its evolution over time. With enough evidence, it is sometimes possible to deduce the origin and approximate date of the original letter in a chain, even if that letter was never found.

Analogous and homologous traits

In examining the traits of organisms in order to put them into their appropriate clades, thorough and systematic studies of their characteristics must be undertaken. Two types of characteristic that are considered are homologous characteristics and analogous characteristics.

As we saw earlier in this chapter, homologous characteristics are ones derived from the same part of a common ancestor. The five-fingered limbs found in such diverse animals as humans, whales, and bats are examples of homologous anatomical structures. The shape and number of the bones may vary, and the function may vary, but the general format is the same, and the conclusion is that the organisms that possess these limbs had a common ancestor.

What do the sarcastic fringehead fish and the bald eagle have in common? Eyes: a homologous characteristic.

Another example of a homologous characteristic is the presence of eyes. Such structures are seen in both vertebrates and invertebrates. Simple eyes found in molluscs such as the *Nautilus* function as pinhole cameras without a system of lenses, whereas highly evolved eyes like those of birds of prey use crystalline lenses, adjustable irises, and muscles to help focus on objects at different distances. Yet both types of eye have evolved from a common ancestor, because they all use one form or another of pigment cells and specialized nerve cells called photoreceptors that are light sensitive (see Chapter 12, Section A.3).

Homology is observed in DNA sequences as well. Certain combinations of base pairs coding for similar proteins can be found in diverse organisms. As with homologous anatomical features, these sequences are evidence of a common ancestry. The cytochrome *c* sequence studied in Section 3.1 is one example.

In contrast, analogous characteristics are those that may have the same function but they do not necessarily have the same structure and they are not derived from a common ancestor. Wings used for flying are an example: eagles, mosquitoes, bats, and extinct reptiles such as the pterosaurs all use (or used) wings to fly. Although these organisms are all classified in the animal kingdom, they are certainly not placed in the same clade simply because of their ability to fly with wings. There are many other characteristics that must be considered.

Another example of an analogous characteristic is fins in aquatic organisms. Both sharks and dolphins have pectoral fins that serve a very similar function: helping them to swim well. But sharks are fish whereas dolphins are aquatic mammals, and the two are classified differently in both the Linnaean system and in cladistics.

To help you remember the difference between analogous and homologous, remember that these terms refer to anatomy (the flesh and blood) and that an analogy is used to compare very different things. The term 'homo' means same, so homologous refers to anatomically similar things.

Cladograms

To represent the findings of cladistics in a visual way, a diagram called a cladogram is used. A cladogram showing bats, sharks, and dolphins, for example, would take into account their skeletal structures and other characteristics, such as the fact that bats and dolphins are mammals (see Figure 5.17). Thus, bats and dolphins are shown as more similar to each other than sharks are to either.

Figure 5.17 shows some key characteristics of a cladogram. For example, a node is the place where a speciation happened and where the common ancestor was found. The clade shown in yellowy green is divided up into a sister group, a group showing the closest relatives, and an outgroup, which is a group that is less closely related to the others in the cladogram. Sharks are less closely related to bats and dolphins than bats and dolphins to each other. And yet, if we go back far enough, we will find another node showing that they do eventually have a common ancestor.

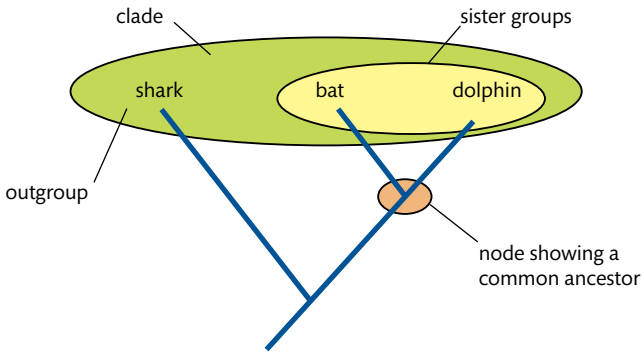


Figure 5.17 A cladogram showing three taxa organized into a clade, of which two are sister groups and one is an outgroup. Nodes show a common ancestor for the descendants that appear above them in this cladogram.

Worked example

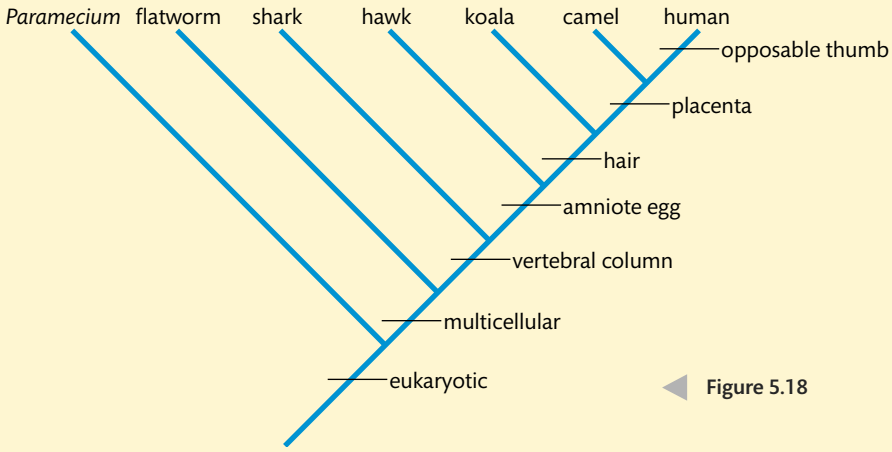


Figure 5.18

1 What is the primitive characteristic in the cladogram shown in Figure 5.18?
2 Name the members of the mammal clade in this cladogram.
3 What is the outgroup when considering the clade of multicellular organisms?
4 Do shark eggs have a protective membrane (the amnios) around them?
5 Explain why there are no bacteria shown in this diagram.

Solutions

1 Being eukaryotic is the primitive characteristic shared by all.
2 Koala, camel, human.
3 The *Paramecium*.
4 No. Sharks are not amniotes.
5 Because the primitive characteristic requires the organisms to have a nucleus. If bacteria were to be added to this cladogram, a new primitive characteristic would need to be chosen.

The essential idea behind cladograms constructed by studying biochemical differences is that an organism with the fewest modifications of a particular DNA sequence will be the most anciently evolved, and those with the most modifications (mutations) in the same DNA sequence will be the more recently evolved organisms. The former have nodes at the earliest splits of the cladogram, and the latter have nodes at the more recent splits.

Cladograms and classification

Cladistics attempts to find the most logical and most natural connections between organisms in order to reveal their evolutionary past. Cladistics is the study of clades, and cladograms are the diagrams that show the phylogeny of the clades being studied.

Every cladogram drawn is a working hypothesis. It is open for testing and for falsification. On the one hand, this makes cladistics scientific, but on the other hand, if it is going to be changing in the future as new evidence arises, it could be criticized for its lack of integrity.

Each time a derived characteristic is added to the list shared by organisms in a clade, the effect is similar to going up one level in the traditional hierarchy of the Linnaean classification scheme. For example, the presence of hair is part of what defines a mammal, so any species found after the line marked 'hair' should be in the class of mammals.

What about feathers? If an organism has feathers, is it automatically a bird? In traditional Linnaean classification, birds occupy a class of their own, but this is where cladistics comes up with a surprise. When preparing a cladogram, it becomes clear that birds share a significant number of derived characteristics with a group of dinosaurs called the theropods. This suggests that birds are an offshoot of dinosaurs rather than a separate class of their own.

Because birds are one of the most cherished and well-documented classes of organisms on Earth, this idea, when it was first suggested, was controversial to say the least. Some of the derived characteristics used to put birds and dinosaurs in the same clade are:

- a fused clavicle (the 'wishbone')
- flexible wrists
- hollow bones
- a characteristic egg shell
- the hip and leg structure, notably with backward-pointing knees.

By following the idea of parsimony, it is more likely that birds evolved from dinosaurs than from another common ancestor. This is where cladistics is clearer than the Linnaean system. In cladistics, the rules are always the same concerning shared derived characteristics and parsimony. In the Linnaean system, apart from the definition of species, which we have already seen is sometimes challenged, the other hierarchical groupings are not always clearly defined: what makes a class a class, or a phylum a phylum? Centuries after Linnaeus, we are still debating this question today.

Reclassification

From time to time, new evidence about a taxon requires a new classification. Either the taxon can be moved up or down the hierarchy (family to subfamily, for example), or from one family to another.

Plants commonly known as figworts used to be classified in the family Scrophulariaceae, and many of them have been used in herbal medicine. The name Scrophulariaceae, sometimes affectionately referred to by botanists as 'scrophs', comes from the time when plants were frequently named for the diseases they could be used

to treat. The medical term 'scrofula' refers to an infection of the lymph nodes in the neck. Preparations made with figwort were given to patients who suffered from this infection, which was associated with tuberculosis.

Before the mid-1990s, the family Scrophulariaceae was characterized by morphological features such as how the flower petals were arranged in the bud before the flower opens. This feature is called aestivation, and botanists look for whether the flower petals overlap with each other or whether they are arranged in a spiral or not. Another characteristic that was used was the morphology of the nectaries, the parts of the flower that make nectar.

Since the mid 1990s, DNA analysis of the plants classified in this taxon have led botanists to rethink their classification. Analysis of zones of DNA markers such as the nuclear ribosomal internal transcribed spacer (ITS) region has revealed that the old classification system was not monophyletic, meaning the taxa did not share a most recent common ancestor. Rather, the old system was grouping together plants that belonged to separate branches, making it impossible to fit them into a cladogram.

The common foxglove, *Digitalis purpurea*, has been reclassified, so instead of being in the figwort family it is now in the plantain family.

The term used to describe species on separate branches is paraphyletic, so we now know that the old family Scrophulariaceae was paraphyletic. As an analogy, it would be similar to someone meeting your extended family for the first time and incorrectly assuming that your second cousins were your brothers and sisters, simply because you all had similar physical features. DNA testing would clearly show that second cousins have a more distant common ancestor than siblings do.

Plants that were in the Scrophulariaceae family have been given new families to belong to. One of the families that has incorporated species from the old classification is the family Plataginaceae, and that is where we now find foxgloves. Foxgloves are now classified in a way that shows that they are more closely related to plantains; they are no longer considered to be figworts.

Moving the branches of the tree of life around and reclassifying a taxon in a new branch in this manner means changing the species' circumscription. Circumscription is the process of placing taxa where they clearly show monophyletic groups, allowing us to show that they all share a recent common ancestor.

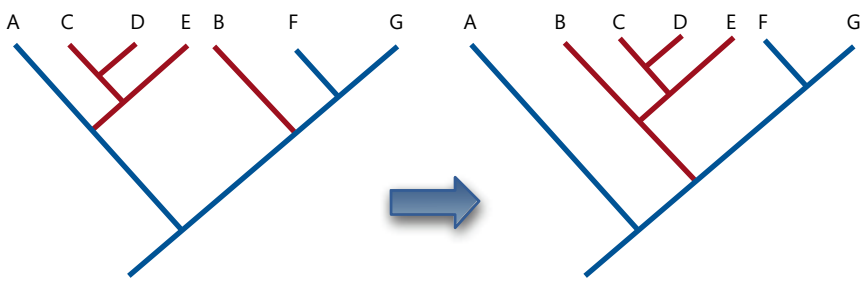


Figure 5.19 An example of a modification of a species' circumscription. The clade that included species C, D, and E on the left was moved from the branch that included species A, and placed on the branch with species B instead, because C, D, and E show a common ancestry with species B. In the old cladogram on the left, B, C, D, and E are shown as being paraphyletic, whereas the new cladogram on the right is showing them as monophyletic.

TOK

Every once in a while a new idea comes along and shakes the scientific community to the core. Reclassifying thousands of organisms by creating a new category of taxon would be a good example, and that is precisely what Carl Woese did in 1977. He proposed the domain Archaea.

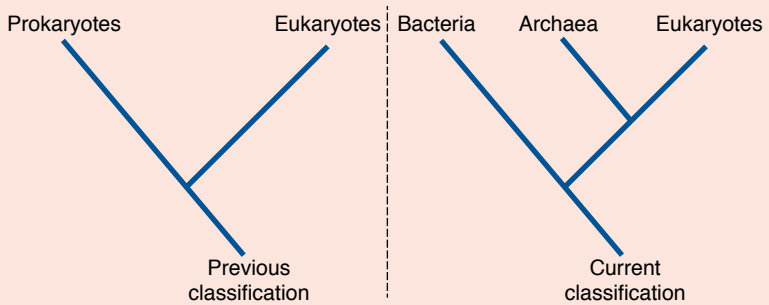


Figure 5.20 The classification of Archaea.

Influential scientists at the time, including Nobel laureate Salvador Lurid and eminent evolutionary biologist Ernst Mayr, opposed splitting the prokaryotes in this way. This is an illustration of how some scientists are conservative and prefer to keep things the way they are. What benefits does conservatism have in science?

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Notice how the reclassification of the foxglove is a good example of how scientists work. Observations were made initially based on morphology. The plant was classified into specific categories that included the family Scrophulariaceae, the figwort family. DNA sequencing was done on many species including foxgloves, and it was determined that some plants did not belong with the other figworts but instead belonged in the family Plantaginaceae along with the plantains. Studies were published in recognized botany journals and now foxgloves have a new family.

A certain amount of communication is needed in order to get everyone to use the new classification. Books on botany and websites on plant conservation, as well as university courses and online databases, must be updated, and the best ones make sure they are backwards compatible (making reference to the previous classification) and forwards compatible (incorporating the latest classification). Not everyone was happy about putting foxgloves with plantains, because visually the plants do not appear to have much in common. But nature is often counterintuitive. If things were obvious in nature, we wouldn't need science to understand it.

Worked example

1 Examine this cladogram of four genera of plants.

(a) Name two sister taxa.

(b) Name the outgroup in this cladogram.

(c) Using a clearly marked label, indicate a node.

(d) Which genus possesses characteristics that evolved more recently, *Digitalis* or *Plantago*?

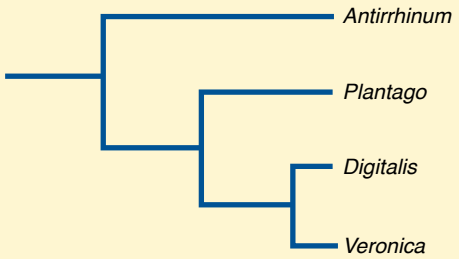


Figure 5.21

2 Study the phylogenic tree below showing some primates and their chromosome numbers. Note that when there is great variety between one species and another within a taxon, a range of chromosome numbers is given.

(a) Identify the numbered arrow that indicates a common ancestor for all the primates shown.

(b) Monkeys have tails whereas apes do not. Arrow number 3 shows the point when primates lost their tails. List the apes shown in the diagram.

(c) Identify the numbered arrow that indicates when bipedalism completely replaced walking on four legs.

(d) The great apes are the four primates shown that demonstrate the most recently developed derived traits. Identify which taxon in the diagram represent the lesser apes.

(e) All the great apes shown except one has the same number of chromosomes? Which species has a different number?

(f) Some evidence supports the idea that, in humans, two of our chromosomes fused together at some point in our evolution. What evidence is there in the cladogram to support this?

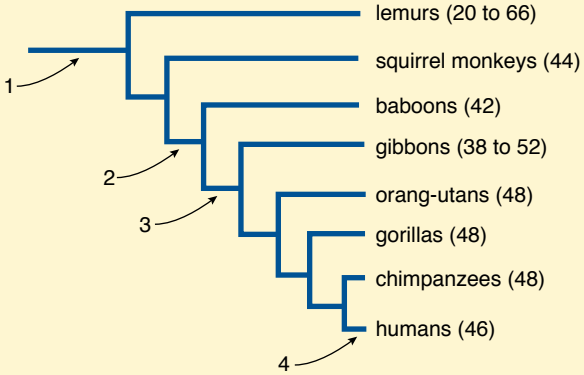


Figure 5.22

Solutions

1 **(a)** *Digitalis* and *Veronica*.

(b) *Antirrhinum*.

(c) Answers may vary: anywhere a horizontal line comes to a 'T' with a vertical line.

(d) *Digitalis* (it is the product of a more recent speciation).

2 **(a)** 1.

(b) Gibbons, orang-utans, gorillas, chimpanzees, humans.

(c) 4.

(d) Gibbons.

(e) Humans.

(f) All of our closest relatives in the great apes clade have 48 chromosomes whereas we have 46; this would suggest that, if one pair of chromosomes fused with another, we would have gone from 24 pairs to 23 pairs.

Exercises

14 Distinguish between analogous and homologous structures.

15 Observe the three amino acid sequences below showing amino acids 100 to 116 in one of the polypeptides that makes up haemoglobin. Next to the human's sequence are two other species, A and B.

Amino acid	Human	Species A	Species B
100	PRO	PRO	PRO
101	GLU	GLU	GLU
102	ASN	ASN	ASN
103	PHE	PHE	PHE
104	ARG	LYS	ARG
105	LEU	LEU	LEU
106	LEU	LEU	LEU
107	GLY	GLY	GLY
108	ASN	ASN	ASN
109	VAL	VAL	VAL
110	LEU	LEU	LEU
111	VAL	VAL	ALA
112	CYS	CYS	LEU
113	VAL	VAL	VAL
114	LEU	LEU	VAL
115	ALA	ALA	ALA
116	HIS	HIS	ARG

(a) How many differences are there between the human sequence and the sequence of species A?

(b) How many differences are there between the human sequence and the sequence of species B?

(c) One of the sequences belongs to a horse and the other to a chimpanzee: which is species B more likely to be? Justify your answer.

Practice questions

1 Which of the following are used as evidence for evolution?

I. Homologous structures.

II. Selective breeding of domesticated animals.

III. Overproduction of offspring.

A I and II only.

B I and III only.

C II and III only.

D I, II and III. (*Total 1 mark*)

2 Outline the process of adaptive radiation. (*Total 3 marks*)

3 What is the mechanism of natural selection?

A Any individuals in a population can be selected entirely by chance.

B After a change in the environment a species will evolve adaptations to the new conditions.

C If an adaptation to the environment is useful, an individual will develop it and pass it on to its offspring.

D Variations amongst individuals of a population are selected by a changing environment.

(*Total 1 mark*)

4 Antibiotic resistance in bacteria is an example of evolution in response to environmental change. Using another example, explain how an environmental change can lead to evolution.

(*Total 8 marks*)

5 What are *Allium sativa* and *Allium cepa*?

A Two different species of the same genus.

B The same species of the same genus.

C The same species but of a different genus.

D Two different species of a different genus. (*Total 1 mark*)

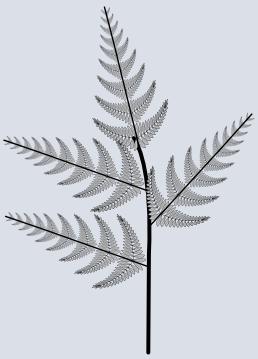
6 Which phylum does the plant below belong to?

A Angiospermophyta.

B Bryophyta.

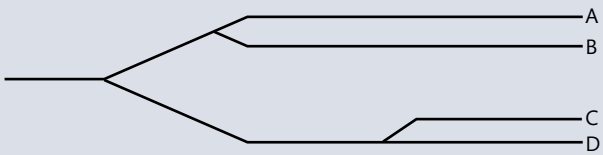
C Coniferophyta.

D Filicinophyta.



(*Total 1 mark*)

7 The cladogram below shows the classification of species A to D. Deduce how similar species A is to species B, C, and D.



(*Total 2 marks*)

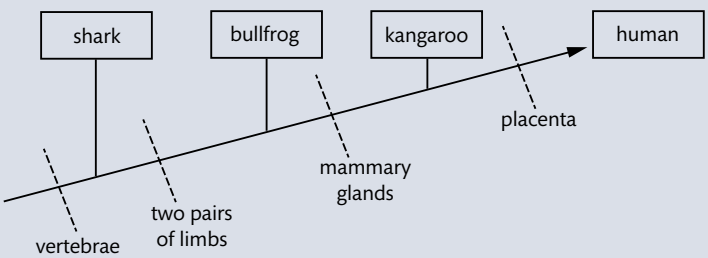
8 Using examples, distinguish between analogous characteristics and homologous characteristics.

(*Total 4 marks*)

9 Suggest two reasons for using cladograms for the classification of organisms.

(*Total 2 marks*)

10 Analyse the relationship between the organisms in the following cladogram.



(*Total 3 marks*)

06 Human physiology

Artwork showing fertilization. Three sperm are shown, but only one will fertilize the ovum.

Essential ideas

6.1	The structure of the wall of the small intestine allows it to move, digest, and absorb food.
6.2	The blood system continuously transports substances to cells and simultaneously collects waste products.
6.3	The human body has structures and processes that resist the continuous threat of invasion by pathogens.
6.4	The lungs are actively ventilated to ensure that gas exchange can occur passively.
6.5	Neurones transmit the message, synapses modulate the message.
6.6	Hormones are used when signals need to be widely distributed.

The human body is composed of cells organized into tissues, tissues organized into organs, and organs organized into organ systems. The anatomy and physiology of the human body is so complex that researchers will be investigating it for many decades to come. In this chapter, you will learn about the physiology of some of the major organ systems of the body, and how those organ systems interact with each other. The science of anatomy is based on identifying structures and parts of structures. The focus of our study will be physiology, which is how the various organs and tissues within your body function. It is a fascinating story.

6.1 Digestion and absorption

NATURE OF SCIENCE

Use models as representations of the real world: dialysis tubing can be used to model absorption in the intestine.

Understandings:

- The contraction of circular and longitudinal muscle of the small intestine mixes the food with enzymes and moves it along the gut.
- The pancreas secretes enzymes into the lumen of the small intestine.
- Enzymes digest most macromolecules in food into monomers in the small intestine.
- Villi increase the surface area of epithelium over which absorption is carried out.
- Villi absorb monomers formed by digestion as well as mineral ions and vitamins.
- Different methods of membrane transport are required to absorb different nutrients.

Applications and skills:

- Application: Processes occurring in the small intestine that result in the digestion of starch and transport of the products of digestion to the liver.
- Application: Use of dialysis tubing to model absorption of digested food in the intestine.
- Skill: Production of an annotated diagram of the digestive system.
- Skill: Identification of tissue layers in transverse sections of the small intestine viewed with a microscope or in a micrograph.

Guidance

- *Students should know that amylase, lipase, and an endopeptidase are secreted by the pancreas. The name trypsin and the method used to activate it are not required.*
- *Students should know that starch, glycogen, lipids, and nucleic acids are digested into monomers, and that cellulose remains undigested.*
- *Tissue layers should include longitudinal and circular muscles, mucosa and epithelium.*

An artist's drawing of the ventral view of a healthy digestive system.

Digestion is an enzyme-facilitated chemical process

When you eat a snack or meal, a series of events is begun that leads to your body cells being provided with the nutrients that they need. Put very simply, the order of events is:

- ingestion – you eat the food
- digestion – a series of chemical reactions occurs, whereby the ingested food is converted into smaller and smaller molecular forms
- absorption – small molecular forms are absorbed through the cells of your digestive system and pass into nearby blood or lymphatic vessels
- transport – your circulatory system delivers the small molecular nutrients to your body cells.

Many of the foods we ingest have very large molecules that are too large to pass across any cell membrane. Yet to get into our bloodstream, molecules must pass through the cell membranes of our intestines and then through the cell membrane of a capillary vessel. Therefore any food that we eat must be chemically digested to a suitable size. Table 6.1 shows different types of molecules found in food and their molecular form before and after digestion.

Table 6.1 Food molecules

Molecule type	Molecular form ingested	Molecular form after digestion
Proteins	Proteins	Amino acids
Lipids	Triglycerides	Glycerol and fatty acids
Carbohydrates	Polysaccharides, disaccharides, monosaccharides	Monosaccharides
Nucleic acids	DNA, RNA	Nucleotides

When we digest food molecules, we hydrolyse them into their smallest components (as shown in the right-hand column of Table 6.1). The components can then be reassembled into larger molecules (macromolecules) that are useful to our bodies.

Humans are incapable of digesting cellulose, one of the most common organic substances on Earth. In fact very few living organisms are capable of digesting cellulose, because they can't produce the enzyme cellulase.

Role of enzymes during digestion

As food moves through your alimentary canal, many digestive enzymes are added to it along the way. Each digestive enzyme is specific for a specific food type. For example, lipase is an enzyme specific for lipid molecules, and amylase is specific for amylose (otherwise known as starch). As you may remember, enzymes are protein molecules that act as catalysts for reactions. As catalysts, the function of enzymes is to lower the activation energy of the reactions that they catalyse. This means that reactions taking place with an enzyme can occur with a lower input of energy than the same reaction

taking place without the presence of an enzyme. The input of energy is typically in the form of heat. Enzyme-catalysed reactions proceed at higher reaction rates at a lower temperature than the same reaction without an enzyme. The reactions of digestion are all very similar because they are all hydrolysis reactions.

Humans maintain a stable body temperature of 37°C. This temperature is warm enough to maintain a good molecular movement and, with the aid of enzymes, it provides enough activation energy for metabolic reactions to occur, including digestion.

Warm-blooded organisms such as humans have an advantage over cold-blooded organisms for efficient digestion and many other metabolic processes, because of their constantly warm internal temperature. However, we would not be able to obtain sufficient nutrients from ingested foods without the aid of digestive enzymes.

Some digested molecules are absorbed into a system of your body called the lymphatic system. This is particularly true of fatty acids because of their non-polarity and relatively large molecular size.

The anatomy of the human digestive system

The human digestive system is fundamentally a long tube called the alimentary canal with two accessory organs (the pancreas and liver) that are connected by ducts into the canal. The alimentary canal begins with the mouth and ends with the anus. Any solids or liquids that you ingest are either, after digestion, absorbed into the bloodstream or, if not absorbed, eliminated as faeces.

The human digestive system shown in Figure 6.1 has been simplified so that you can use it as a basis for practising drawing and labelling the digestive system. The lungs are shown to give some perspective to the location of the thoracic cavity, which contains the heart and lungs, compared with the abdominal cavity, which contains all of the digestive structures shown apart from the mouth and oesophagus.

Make sure you practise the drawings that you will be expected to be able to produce in an exam. Adding labels to an existing diagram is relatively easy compared with starting from a blank piece of paper and producing an entire diagram with labels and/or annotated functions.

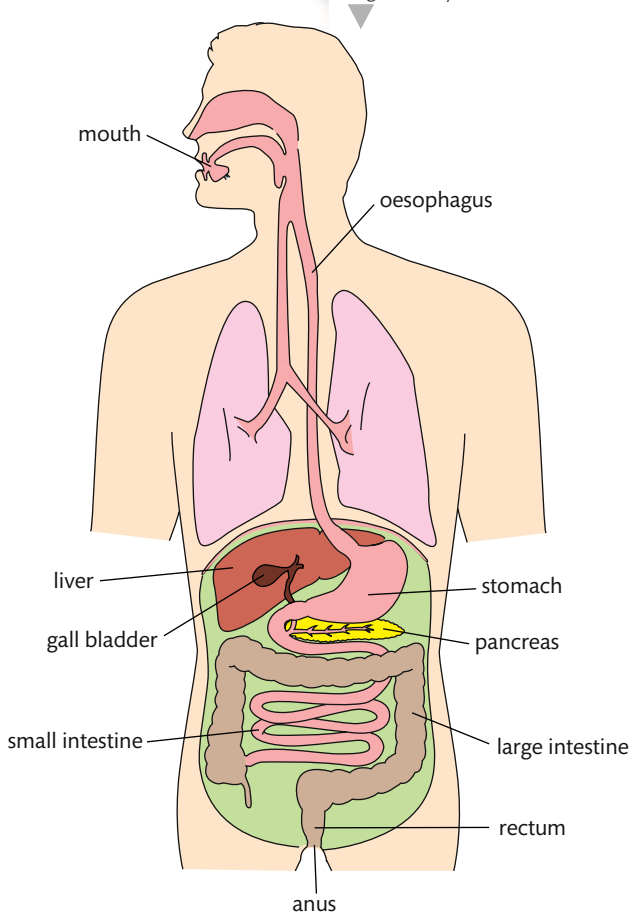


Figure 6.1 The human digestive system.

The alimentary canal is a muscular tube

Food does not make its one-way journey through the alimentary canal by gravity. Indeed, food material often has to move against gravity. So, what keeps food moving, and moving in the one direction? The answer is muscles, specifically smooth muscles. Smooth muscle is controlled by the autonomic nervous system (ANS), and you are not aware that your smooth muscle is contracting. The tube of the alimentary canal has two layers of smooth muscle, called circular and longitudinal. A simplified drawing of these two layers is shown in Figure 6.2. The contracting fibres of the inner, circular, muscles do indeed make a 'circle', as shown in this section, while the contracting fibres of the longitudinal muscles are positioned at right angles to the circular muscles. The muscle motion and food movement caused by the action of these two muscle layers is called peristalsis.

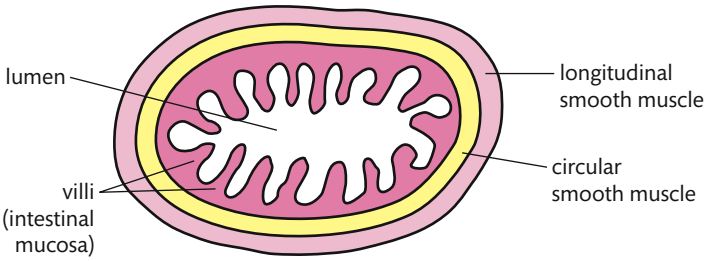
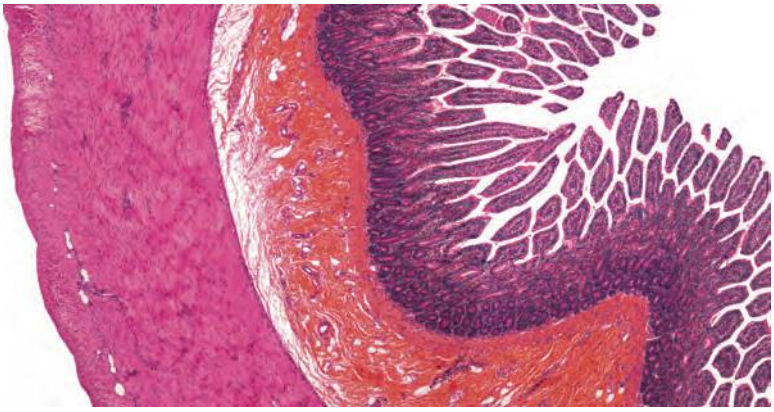


Figure 6.2 This simplified drawing shows a section of the small intestine showing the relative locations of the circular and longitudinal muscles. The same arrangement would also be found in the oesophagus, stomach, and large intestine. The only part of this sketch that is specific to the small intestine is the villi, used for absorption.



A light microscope photograph showing a small area of the small intestine. The white area in the upper right corner is the lumen (cavity) of the intestine, where unabsorbed food would be located. To the lower left of that are the villi, which are used for absorption. Further left are the circular and longitudinal muscle layers used for peristalsis.

Peristalsis is used in the stomach to mix food with digestive secretions, including a protein-digesting enzyme. This movement is called churning. In the rest of the alimentary canal, peristalsis causes a contraction just 'behind' the food mass and thus keeps it moving through the canal, as well as helping to mix the food with a variety of enzymes. The peristaltic movement is relatively fast within the oesophagus and slows dramatically in the intestines.

A person who is hanging upside down can still swallow food and the food will travel 'up' to the stomach. This is because the food is moved by peristalsis, not by gravity.

The role of the pancreas during digestion

The pancreas is a multipurpose organ. In addition to producing two important hormones (insulin and glucagon) involved in glucose metabolism, the pancreas produces three enzymes involved in digestion: lipase, amylase, and a protein-digesting enzyme known as an endopeptidase. Those three enzymes are part of a fluid known simply as pancreatic juice that is released into the first portion of the small intestine through a duct. Look closely at the artwork opposite and you will see the pancreatic duct. The duct allows the three enzymes to enter the lumen (cavity) of the small intestine, where partially digested food from the stomach is being released.

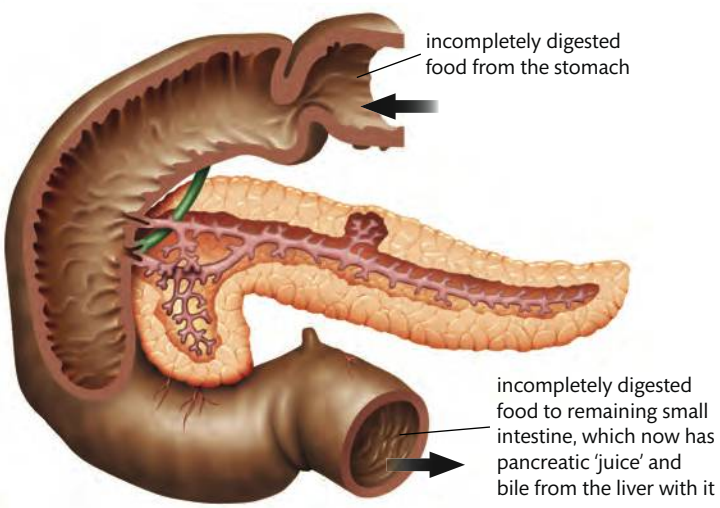
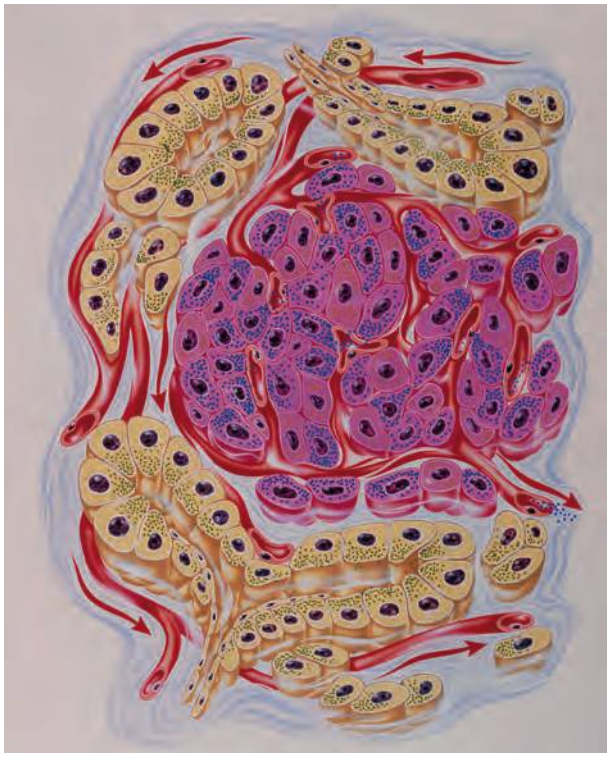


Figure 6.3 Artwork showing the pancreas and pancreatic ducts leading to the lumen of the first section of the small intestine. The green tube shown is bringing bile from the liver (not shown) to be added to aid lipid digestion. The area at the top that is cut is where the stomach is located, and the lower area that is cut is where the very long small intestine continues.

Table 6.2 Digestive enzymes produced by the pancreas and secreted into the lumen of the small intestine

Enzyme	Substrate	Action
Lipase	Lipids (fats and oils)	Hydrolyses lipids into glycerol and fatty acids
Amylase	Starch	Hydrolyses starch into the disaccharide maltose. Another enzyme then hydrolyses maltose into glucose
Trypsin (an endopeptidase)	Proteins (polypeptides)	An endopeptidase hydrolyses long polypeptides into smaller polypeptides. Further protein-digesting enzymes then hydrolyse the smaller polypeptides into amino acids



This illustration shows the cells that are involved in two major aspects of pancreatic function. The brightly coloured cells are endocrine (hormone-producing cells), which produce hormones that are then transported away by the bloodstream. The yellow-coloured cells are cells that produce digestive enzymes that are released into very small ducts (look closely at the lower left of the picture) that eventually join into the pancreatic duct that leads to the small intestine.

The role of the small intestine in digestion and absorption

As an example of what happens as ingested foods move through the small intestine, let's see how starch is digested and how its monomers are absorbed.

The chemical digestion of starch begins in the mouth, with the addition of saliva to the food. Saliva contains amylase, the enzyme that hydrolyses the starch polysaccharide into the disaccharide maltose. The hydrolytic activity of amylase ceases in the highly acidic environment of the stomach. Therefore, the starch remains largely undigested when the contents of the stomach are released into the small intestine.

As described earlier, the pancreas produces and secretes pancreatic juice, and sends that juice into the first section of the small intestine, which is called the duodenum. One of the components of pancreatic juice is amylase. The pH environment of the small intestine is neutral to slightly alkaline, which is the optimum pH for amylase. Thus the amylase molecules begin to catalyse the hydrolysis of starch to maltose. As peristalsis continues to move the food through the lumen of the small intestine, the hydrolytic reactions continue.

Within the small intestine there is another enzyme that completes the digestion of starch. The enzyme maltase catalyses the hydrolysis of maltose into two molecules of glucose. Maltase is produced by the cells of the inner lining of the small intestine, and typically remains bound into the plasma membranes of the epithelial cells that are in contact with the food material within the lumen.

Absorption of glucose into villi

The cells in the inner lining of the small intestine make up what is called the mucosa. The mucosa has many small folds or projections called villi (singular villus). Each villus is composed of many cells whose primary job is selectively absorbing molecules found in the lumen of the small intestine. The actual absorption occurs through cells in an epithelial layer that is in direct contact with the nutrients. The epithelial cells have tiny

The hydrolytic enzyme lactase is being extracted commercially from certain yeasts, and is being used to hydrolyse the disaccharide lactose from milk and milk products. This is especially helpful for people who are lactose intolerant.

membrane projections called microvilli that extend into the lumen of the intestine. The villi and microvilli greatly increase the surface area for absorption within the small intestine, compared with a smooth-walled structure. The interior of each villus contains a capillary bed for nutrient absorption and transport of digested monomers by the bloodstream. In addition, there is a small vessel of the lymphatic system present, called a lacteal, that absorbs some of the nutrients. After passing through the epithelial cells of a villus, most monomers are absorbed into the inner capillary bed. However, some of the larger monomers, such as fatty acids, are absorbed first into a lacteal.

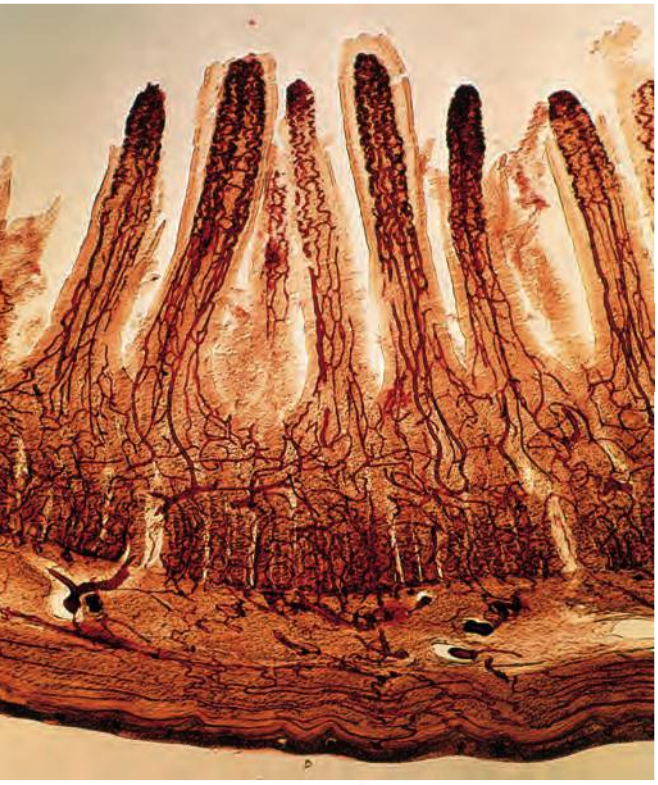
Here is a partial list of the substances absorbed through villi into the bloodstream or lymph fluid:

- water
- glucose (plus other monosaccharides)
- amino acids
- nucleotides
- glycerol
- fatty acids
- mineral ions
- vitamins.

TOK

Each country has its own laws concerning how food is labelled for consumers. Some countries require detailed lists of important information, such as fat content, fat type, calories per serving, etc., while other countries have no requirements at all. Do all the citizens of a country have a right to know the contents of the food that they are buying?

A light microscope photograph showing a transverse section through several villi. Microvilli are too small to be seen at this magnification. The capillary bed inside each villus is clearly visible. The longitudinal and circular muscles of the wall of the intestine are also visible at the bottom of the photograph.



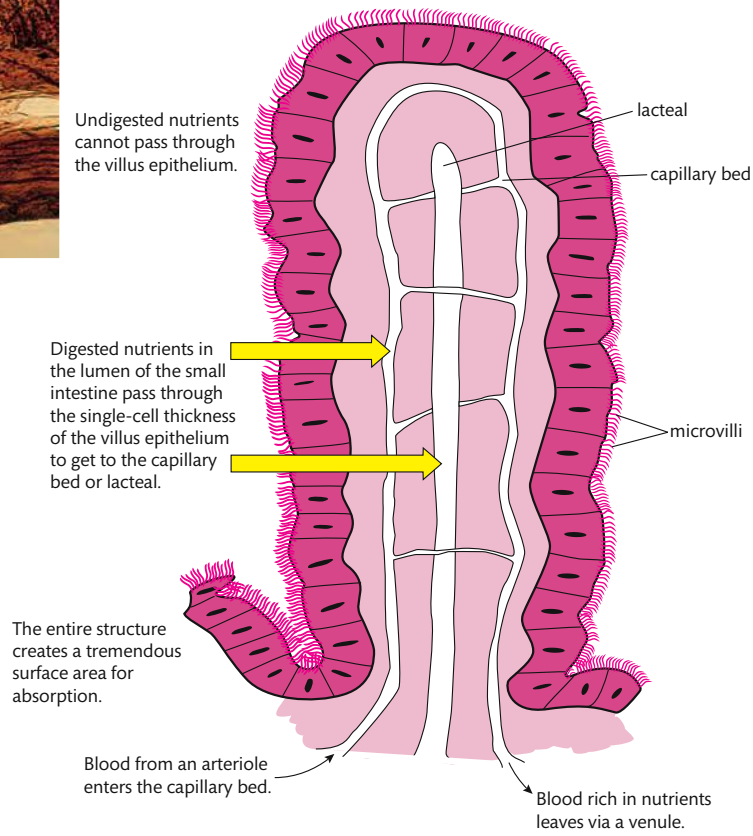


Figure 6.4 The structure of an intestinal villus. It is estimated that each square millimetre of small intestine contains approximately 10–40 villi. Thus the entire small intestine of a human contains millions of villi and even more microvilli.

Transport mechanisms used by epithelial cells to absorb nutrients

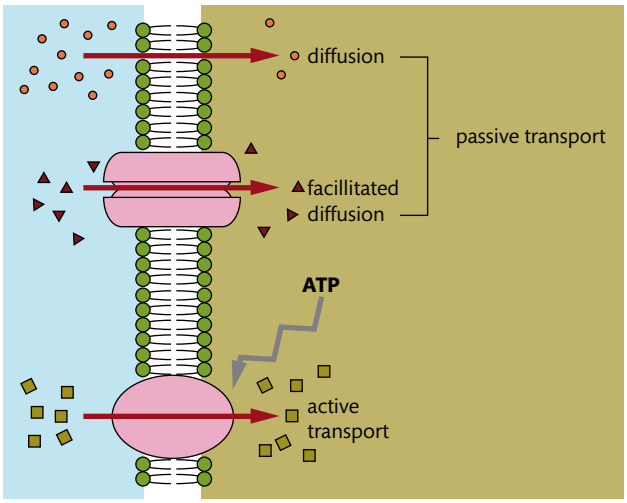


Figure 6.5 Schematic view of three of the more important mechanisms used by cells of the villi epithelium to absorb nutrients from the lumen of the intestine. The mechanism used depends on the size and polarity of the molecule transported. Not shown is endocytosis where a portion of the plasma membrane invaginates to take in many molecules at one time.

A variety of mechanisms are used for nutrient molecules to cross the epithelial layer of the villi mucosa. Here is a summary of some of those transport mechanisms.

Passive mechanisms: no ATP used

- Simple diffusion: direct movement through the cell membrane following a concentration gradient. Examples: very small molecules and non-polar molecules, such as fatty acids, which dissolve through the biphospholipid layer of the membrane.
- Facilitated diffusion: movement through a cell membrane following a concentration gradient, but the molecule must travel through a protein channel because of its size and polarity. Examples: glucose and amino acids.

Active mechanisms: ATP expended

- Membrane pumps: molecules moved against their concentration gradient by certain proteins using ATP to 'pump' the molecule across the membrane. Examples: glucose, and amino acids under certain circumstances.
- Endocytosis (pinocytosis and phagocytosis): molecules are trapped in an invagination (infolding) of the membrane and pass through to the other side of the membrane as a vesicle. Example: some macromolecules that have not yet been fully digested.

Exercises

1. A single sandwich is likely to contain carbohydrates, lipids, and proteins. From a biochemical viewpoint, what happens to each of these types of molecules upon digestion?
2. You ingest a glucose molecule within the starch of a breakfast cereal. List as many locations as you can that this single glucose molecule will visit from the time that it is in your mouth to the time it enters a muscle cell of your body.
3. What role does the pancreas play in the digestive process?

NATURE OF SCIENCE

Theories are regarded as uncertain: William Harvey overturned theories developed by the ancient Greek philosopher Galen on movement of blood in the body.

6.2 The blood system

Understandings:

- Arteries convey blood at high pressure from the ventricles to the tissues of the body.
- Arteries have muscle cells and elastic fibres in their walls.
- The muscle and elastic fibres assist in maintaining blood pressure between pump cycles.
- Blood flows through tissues in capillaries. Capillaries have permeable walls that allow exchange of materials between cells in the tissue and the blood in the capillary.
- Veins collect blood at low pressure from the tissues of the body and return it to the atria of the heart.
- Valves in veins and the heart ensure circulation of blood by preventing backflow.
- There is a separate circulation for the lungs.
- The heart beat is initiated by a group of specialized muscle cells in the right atrium called the sinoatrial node.
- The sinoatrial node acts as a pacemaker.
- The sinoatrial node sends out an electrical signal that stimulates contraction as it is propagated through the walls of the atria and then the walls of the ventricles.
- The heart rate can be increased or decreased by impulses brought to the heart through two nerves from the medulla of the brain.
- Epinephrine increases the heart rate to prepare for vigorous physical activity.

Applications and skills:

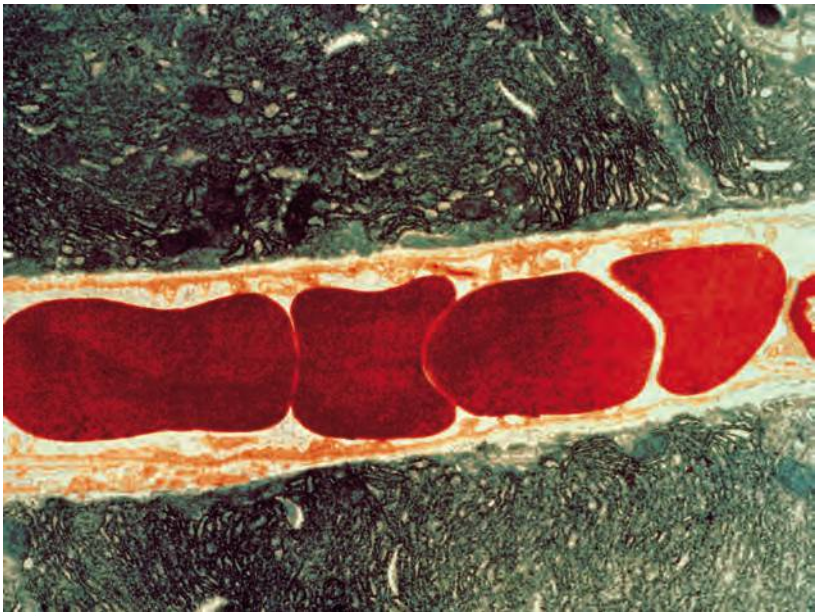
- Application: William Harvey's discovery of the circulation of the blood with the heart acting as the pump.
- Application: Pressure changes in the left atrium, left ventricle, and aorta during the cardiac cycle.
- Application: Causes and consequences of occlusion of the coronary arteries.
- Skill: Identification of blood vessels as arteries, capillaries, or veins from the structure of their walls.
- Skill: Recognition of the chambers and valves of the heart and the blood vessels connected to it in dissected hearts or in diagrams of heart structure.

Arteries, capillaries, and veins

Arteries are blood vessels taking blood away from the heart that has not yet reached a capillary. Veins are blood vessels that collect blood from capillaries and return it to the heart. Identifying a blood vessel as being an artery or a vein has nothing to do with whether the blood is oxygenated or deoxygenated. For example, blood leaving the right ventricle is flowing through pulmonary arteries, even though it needs to be re-oxygenated in the capillaries of the lung tissue. These blood vessels are pulmonary arteries because they are between the heart and the capillary bed. The newly oxygenated blood will be brought back to the heart by the pulmonary veins.

Arteries have a relatively thick, smooth, muscle layer that is used by the autonomic nervous system to change the inside diameter (lumen) of the blood vessels. In addition to smooth muscle, arteries have elastic fibres that help maintain the relatively high blood pressure achieved by the contractions of the ventricles. When blood is pumped into an artery, the elastic fibres are stretched and allow the blood vessel to accommodate the increased pressure. When the contraction is over, the elastic fibres provide another source of pressure as they return to their original position. This helps maintain the blood pressure between pump cycles. Remember that blood in arteries is at a high pressure because arteries are the vessels that are directly connected to the ventricles of the heart. When blood leaves an arteriole (the smallest of the arteries),

it enters a capillary bed rather than a single capillary. A capillary bed is a network of capillaries that typically all drain into a single venule.

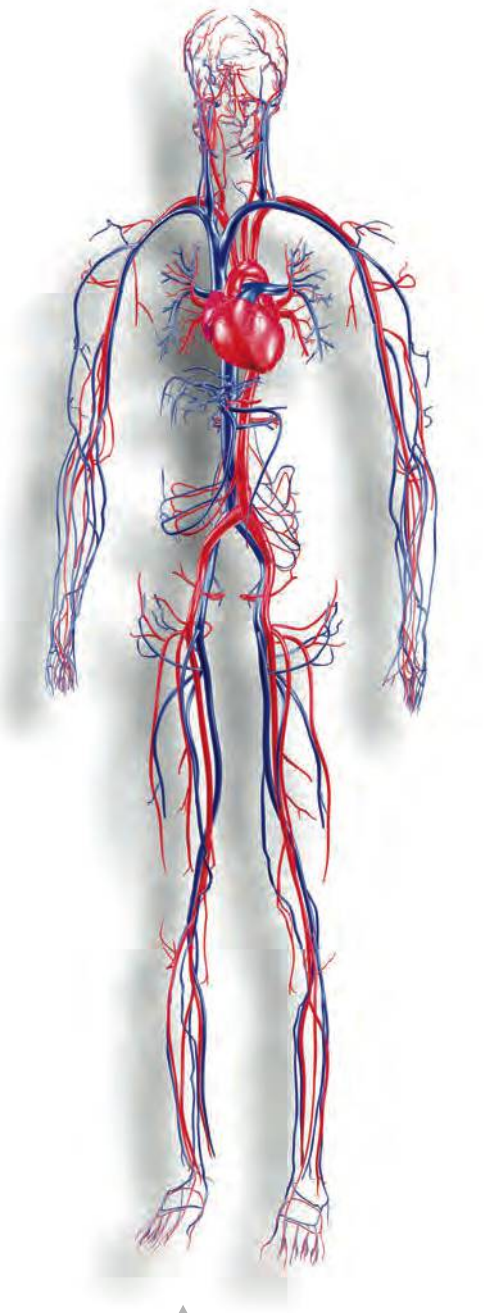


A false-colour transmission electron micrograph (TEM) of a capillary containing erythrocytes (red blood cells). Notice the thin 'wall' of the capillary, which is conducive to the movement of molecules in and out of the bloodstream.

When blood enters a capillary bed much of the blood pressure is lost. Blood cells make their way through capillaries one cell at a time. Chemical exchanges always occur through the single-cell thickness of capillaries, because the walls of arteries and veins are too thick to allow molecules in or out efficiently. Veins receive blood at a relatively low pressure from the capillary beds. Because this blood has lost a great deal of blood pressure, the blood flow through veins is slower than through arteries. To account for this, veins have thin walls and a larger internal diameter. Veins also have many internal passive 'one-way flow' valves that help keep the slow-moving blood travelling consistently towards the heart. Table 6.3 summarizes the three types of blood vessels.

Table 6.3 A comparison of arteries, capillaries, and veins

Artery	Capillary	Vein
Thick walled	Wall is 1 cell thick	Thin walled
No exchanges	All exchanges occur	No exchanges
No internal valves	No internal valves	Internal valves present
Internal pressure high	Internal pressure low	Internal pressure low

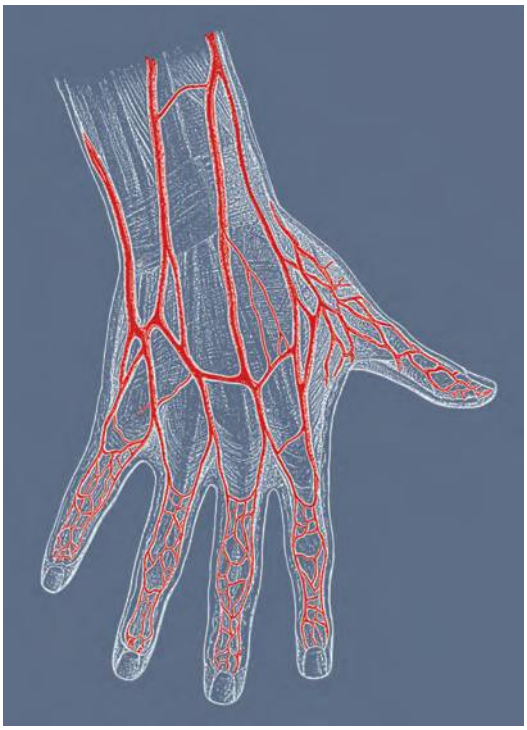


If all the tissue except blood vessels and heart were removed from a body, the shape of the body would still be visible.

Fish have a two-chambered heart and amphibians have a three-chambered heart. Reptiles, birds, and mammals all have a four-chambered heart (although the ventricles of a reptile heart are only partially divided).

The heart, a double pump

The human heart is designed as a pair of side-by-side pumps. Each side of the heart has a collection chamber for the blood that moves in slowly from



A drawing showing some of the larger blood vessels in the hand. Smaller vessels like capillaries cannot be seen without magnification.

the veins. These thin-walled, muscular chambers are called atria. Each side also has a thick-walled muscular pump called a ventricle, which builds up enough pressure to send the blood out from the heart with a force we refer to as blood pressure. This double-sided pump works every minute of every day of your life. The blood that is pumped out from the heart typically makes a circuit through the following sequence of blood vessels:

- a large artery
- smaller artery branches
- an arteriole (the smallest type of artery)
- a capillary bed
- a venule (the smallest type of vein)
- larger veins
- a large vein, which takes the blood back to the heart to be pumped out once again.

The two sides of the heart form two major routes for blood to flow along (see Figure 6.6). The right side of the heart sends blood along a route that is called your pulmonary circulation. Along this route, the capillary beds are found in your lungs, where the blood picks up oxygen and releases carbon dioxide.

The left side of the heart sends blood along a route that is called the systemic circulation. The artery that emerges from the heart at the beginning of this route is the aorta. Branches of the aorta carry blood to almost every organ and cell type in your body. Along this route, the capillary beds are found in your organs and tissues, where the blood picks up carbon dioxide and releases oxygen.

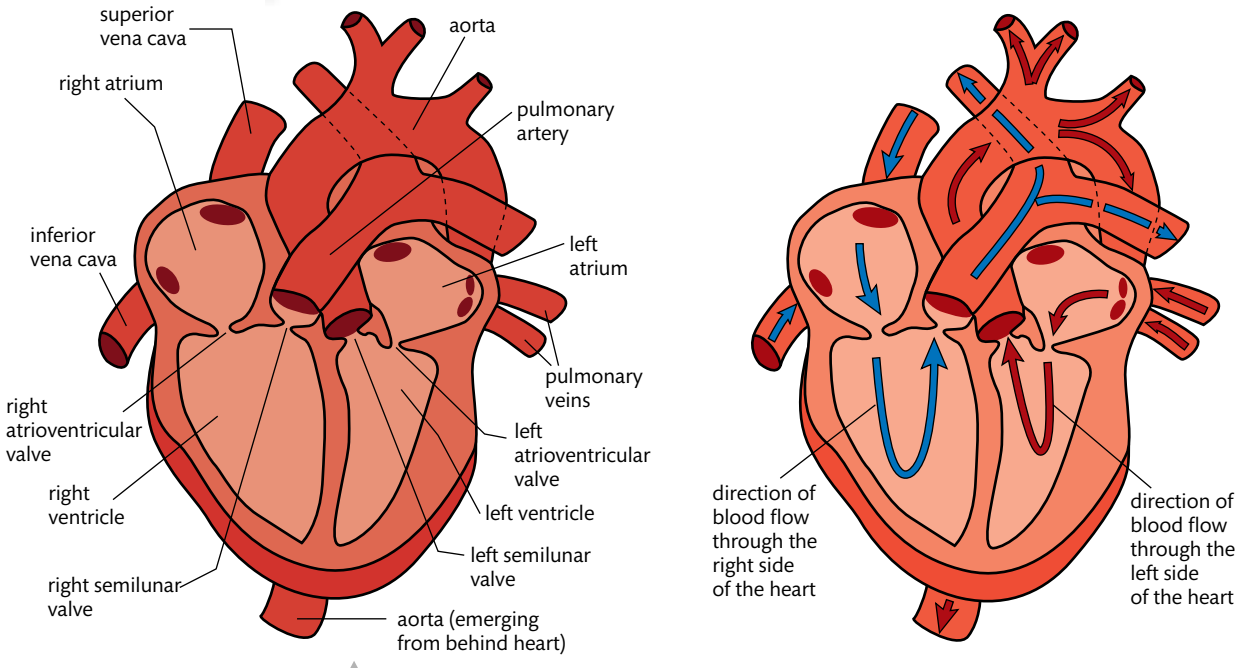


Figure 6.6 Human heart anatomy and blood flow. In the bottom right diagram the blue arrows represent deoxygenated blood and the red arrows represent oxygenated blood.

Control of the heart rate

The majority of the tissue that makes up the heart is muscle. More specifically, it is cardiac muscle. Cardiac muscle spontaneously contracts and relaxes without any control by the nervous system. This is known as myogenic muscle contraction. However, the myogenic activity of the heart does need to be controlled, in order to make the timing of the contractions unified and useful.

Within the right atrium there is a mass of specialized tissue that has properties of both muscle and nervous system cells within its walls; this tissue is called the sinoatrial node (SA node). The SA node acts as the pacemaker for the heart by sending out an 'electrical' signal to initiate the contraction of both atria. For a person with a resting heart rate of 72 beats a minute, the signal from the SA node is sent out every 0.8 seconds. Also within the right atrium is another mass of specialized muscle tissue, known as the atrioventricular node (AV node). The AV node receives the signal from the SA node, delays for approximately 0.1 seconds, and then sends out another 'electrical' signal. This second signal goes to the thick muscular ventricles and results in their contraction. This explains why both atria, and then later both ventricles, contract in synchrony (see Figure 6.7).

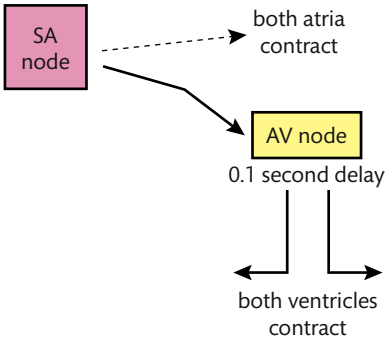
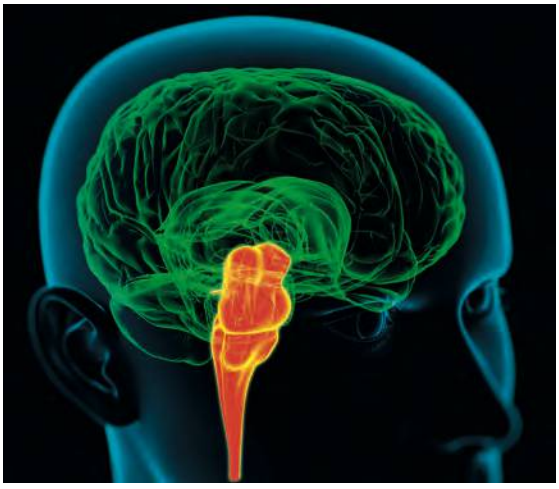


Figure 6.7 Myogenic control of the heart rate. The SA node acts as the pacemaker. The AV node relies on a signal from the SA node to send impulses to the ventricles. Notice the delay between the two events that allows the atria to contract, followed shortly after by the contraction of the ventricles.

During times of increased body activity, such as exercise, the heart rate needs to increase above the resting heart rate. This is because there is an increased demand for oxygen for cell respiration during periods of heavy exercise or activity. There is also a need to get rid of the increased levels of carbon dioxide that accumulate in the bloodstream. As exercise begins and carbon dioxide levels begin to rise, an area of your brainstem called the medulla chemically 'senses' the increase in carbon dioxide.



Much of the orange area of this computer artwork shows an area of the brainstem called the medulla (oblongata). Chemoreceptors in the medulla are sensitive to carbon dioxide changes in the blood as it passes through.

CHALLENGE YOURSELF

1 Study the left part of Figure 6.6 and learn the names of the chambers, valves, and blood vessels associated with the heart. Then cover up the diagram and follow an imaginary red blood cell through the complete circulation pattern as shown in the right part of the figure. When you can do this without any mistakes, cover the right diagram and use the left diagram to trace the blood flow, noting at each location whether the blood is oxygenated or deoxygenated.

The increase in number of chambers of the heart, from the two characteristic of fish to the four characteristic of birds and mammals, allows complete separation of deoxygenated and oxygenated blood. In other words, the evolution of the four-chambered heart led to the separation of the pulmonary and systemic circulations.

As you continue your study of human physiology, look for instances where two or more systems of the body interact in order to accomplish an action. For example, during exercise, your heart rate cannot increase or return to its resting heart rate without the nervous system and the circulatory system interacting.

Computer artwork showing the two kidneys in a male. The lighter coloured tissue on the upper portion of each kidney is an adrenal gland. Like all endocrine glands, adrenal glands secrete their hormone (epinephrine) into the bloodstream for distribution to all parts of the body.

The medulla then sends a signal through a cranial nerve, called the cardiac nerve, to increase the heart rate to an appropriate level. This signal is sent to the SA node; it does not change the mechanism of how the heart beats, just the timing. After exercise, the level of carbon dioxide in the bloodstream begins to decrease and another signal is sent from the medulla. This time the signal is carried by a different cranial nerve, called the vagus nerve. Electrical signals from the vagus nerve result in the SA node once again adjusting the timing of the heart rate, so that the heart returns to its myogenic or resting heart rate.

The heart rate can also be influenced by chemicals. One of the most common is epinephrine (also called adrenaline). During periods of high stress or excitement, your adrenal glands secrete epinephrine into the bloodstream. Among other effects, epinephrine causes the SA node to 'fire' more frequently than it does at its resting heart rate, and thus the heart rate increases, sometimes dramatically so.

CHALLENGE YOURSELF

2 Try to verbalize the events that lead to the following.

- The contraction of both atria followed by contraction of both ventricles for a person who is currently at his or her resting heart rate.
- The increase in heart rate for a person who has recently begun exercising.
- The decrease in heart rate for a person who has recently stopped exercising.

A (single) cardiac cycle is what most people think of as a 'heart beat'. A cardiac cycle is initiated by the SA node impulse and includes all the heart events that follow until another SA node signal begins a new cardiac cycle.

Changes in pressure within the heart chambers keep the blood moving

Heart valves open and close depending on the pressure of the blood on each side of the valve. The change in pressure also explains the movement of blood through and out of each chamber of the heart. Both the left and right sides of the heart work synchronously as a double pump. To understand the workings of the heart, it is only necessary to look at one side of the heart with the understanding that the other side has similar pressures and volumes of blood at the same time.

Let's examine the pressure and volume changes that occur on the left side of the heart. You do not have to memorize the pressure numbers given in this example, your focus should be on understanding how the given blood pressures result in the movement of blood and the opening and closing of the heart valves.

When both chambers are at rest

The term used for a chamber of the heart that is not contracting is diastole. The term used for a chamber of the heart that is contracting is systole. Thus the time period when both chambers are at rest can be described as both chambers undergoing diastole.

Figure 6.8 shows the left side of the heart with openings in the left atrium for entry of the pulmonary veins. The numbers inside each chamber or blood vessel represent the pressure measured in mm Hg. Heart valves open and close based on blood pressure differences on either side of any one valve. During this period of diastole for both chambers, the atrial pressure is just slightly higher than ventricular pressure, and

this keeps the left atrioventricular valve open. Much of the blood that slowly returns to the left atrium via the pulmonary veins moves passively down to the left ventricle through this open valve. Notice also that the pressure in the aorta is much higher than in the left ventricle. This pressure difference keeps the left semilunar valve closed and prevents backflow into the ventricle.

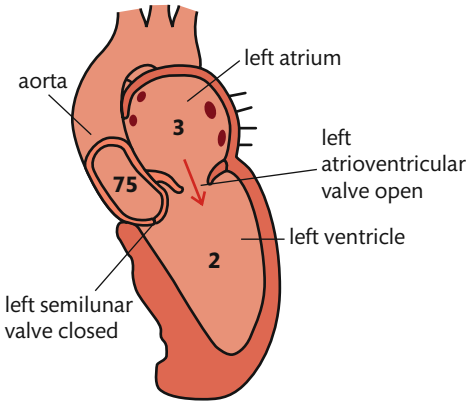
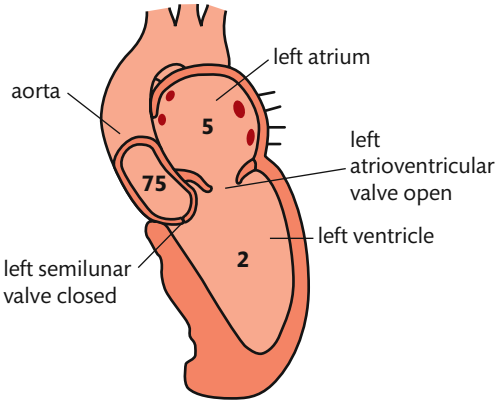


Figure 6.8 Blood pressure readings in mm Hg when both chambers are in diastole (rest). Notice that some blood is moving passively from the left atrium to the left ventricle.

When the atria are in systole and the ventricles are in diastole

In Figure 6.9, the atrium is undergoing a systole (contraction). The pressure produced by this systole is not very high. The wall of each atrium is relatively thin muscle and is not capable of creating very much pressure. There is no need for great pressure because much of the volume of blood has already accumulated passively within the ventricle through the open atrioventricular valve. Any remaining blood in the atrium is moved to the ventricle by the systole.



The muscular walls of both atria are very thin, and the pressure exerted during atrial systole is very low. Conversely, the muscular walls of the ventricles are very thick, and the pressure exerted during ventricular systole is very high.

Figure 6.9 Typical blood pressure readings in mm Hg during atrial systole.

When the atria are in diastole and the ventricles are in systole

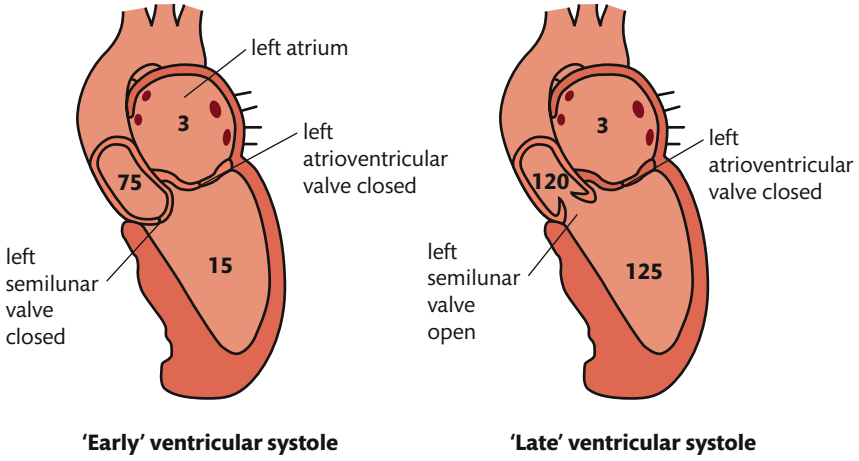


Figure 6.10 Blood pressure readings in mm Hg at early and late ventricular systole.

Figure 6.10 shows the blood pressures in early and late ventricular systole. As soon as ventricular systole begins, the pressure inside the ventricle increases to be greater than that in the atrium, so the atrioventricular valve closes to prevent backflow to the atrium (this creates the 'lub' sound that can be heard with a stethoscope). The pressure in the aorta is still far higher than in the ventricle, so the semilunar valve

Blood pressure is often measured in mm Hg, although the modern units for pressure are pascals (Pa) and kilopascals (kPa).

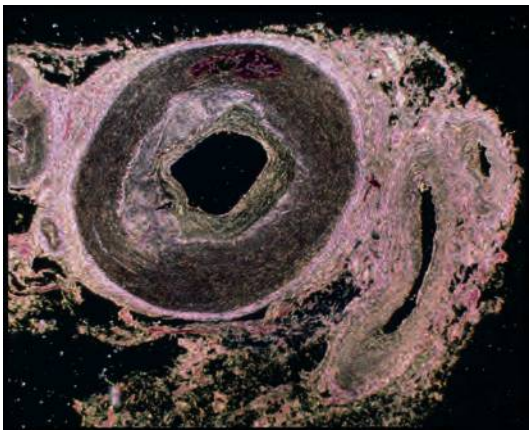
120 mm Hg = 16 kPa

80 mm Hg = 11 kPa

The mean population blood pressure varies widely from country to country. As a general rule, high blood pressure is positively correlated with the consumption of salt and obesity, and is negatively correlated with the consumption of fruits and vegetables.

remains closed. There is a relatively large volume of blood in the ventricle during this time, and the ventricle is highly muscular. This combination of factors permits the ventricular pressure to build up considerably as systole continues. Finally, the pressure in the ventricle becomes greater than that in the aorta, and the semilunar valve opens, allowing the ventricle to pump the blood into the aorta. As the ventricle finishes its contraction, the pressure inside it once again drops below the pressure in the aorta, and the semilunar valve closes (this causes the 'dub' sound that can be heard with a stethoscope). Both chambers go back into diastole and the cardiac cycle repeats itself again, and again.

Build-up of plaque in arteries leads to atherosclerosis



An artery showing artherosclerosis. The dark area in the centre is the lumen, where blood flows. The light grey area surrounding the lumen is plaque. The lumen of this blood vessel is significantly smaller than it was at an earlier time in this person's life.

Atherosclerosis is a slow build-up of materials in the arteries that is collectively called plaque. Plaque is composed of lipids, cholesterol, cell debris, and calcium. The build-up of this material begins early in life and typically takes many, many years to become a serious problem. As arteries begin to build up plaque, they become harder and therefore less flexible. The inside lining of an artery is known as the endothelium. In a young person, the endothelium of each artery is smooth, with no plaque build-up. As the years progress, each person begins to deposit plaque. How much depends on a whole set of factors, with genetics and eating habits being of prime importance.

Occlusion in coronary arteries can lead to a heart attack

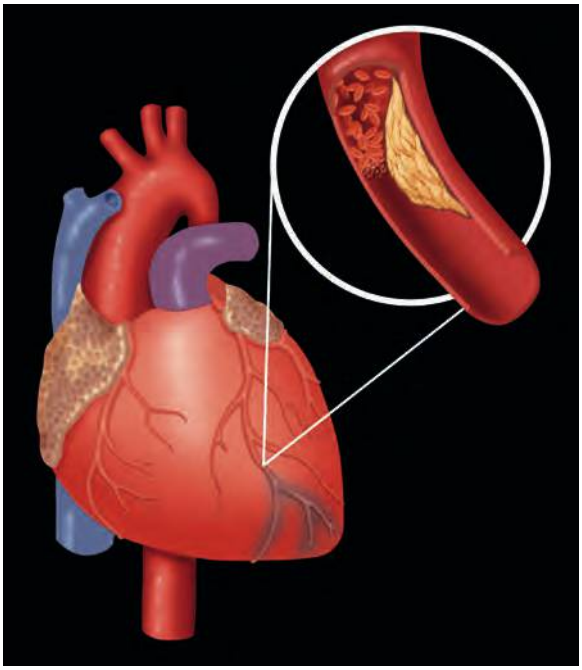


Illustration showing an occlusion (a clot caused by plaque build-up) in a coronary artery.

The heart has three major coronary arteries that supply the heart muscle with oxygen-rich blood. These arteries are branches direct from the aorta and carry blood that has recently been to the lungs. As you will recall, cardiac muscle never stops contracting, with alternating periods of systole and diastole occurring repeatedly throughout your life. Thus cardiac muscle is very oxygen-demanding. If any one of the three major coronary arteries, or one or more of their branches, is somehow blocked, some portion of the heart muscle is likely to be deprived of its oxygen supply. This is exactly what happens when atherosclerosis eventually leads to a partial or complete occlusion. The term occlusion describes the condition when plaque build-up has become so substantial that the blood vessel can no longer supply even a minimally healthy volume of blood to the tissue that it 'feeds'.

When a coronary artery or one of its main branches becomes blocked, it is known as a coronary thrombosis or an acute myocardial infarction, i.e. a heart attack.

NATURE OF SCIENCE

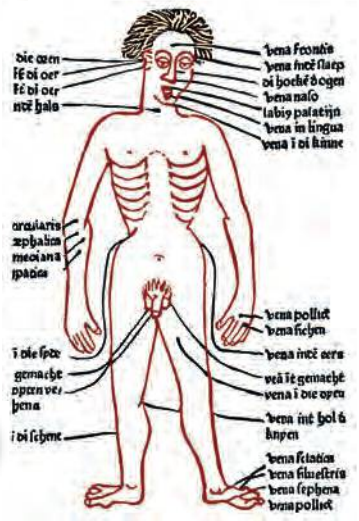
Have you ever thought about how difficult it would be to convince everyone around you that something they and everyone else had been taught and firmly believed in was actually false? Especially something that has been believed for many centuries?

It would be an exceptionally difficult thing to do, and attempting to do this might mean you are considered to be a lunatic.

A man by the name of William Harvey made such an attempt after his experimental work showed how blood is circulated around the body. Prior to Harvey's experimental work, the authority on the movement of blood in the body was provided by the early Greeks (AD 100–200), including Pliny the Elder and Galen (of Pergamon). These Greeks postulated that blood was constantly being used up within the body, and they did not consider the closed circulation pattern we now know exists. Galen taught his students that there were two types of blood: 'nutritive blood' that was made by the liver, and 'vital blood' that was made by the heart and distributed through the arteries to carry the 'vital spirits'. Further, Galen taught his students that blood flowed from one ventricle of the heart to the other through tiny pores. In order to understand the context of Galen's teachings, you must imagine blood that is not flowing through blood vessels as we think of now, but rather seeping slowly from one location to another until the blood in the body is 'used up'. The latter was the thinking of virtually every person trained in medicine for more than 1300 years.

After years of animal dissections, live animal experimentation, and human cadaver dissections, William Harvey determined that the heart acts as a double pump (with systemic and pulmonary circulations), and that the blood is continuously circulated to/from the lungs and to/from the body. He was not able to see the capillaries that connected arteries to veins, but he postulated their existence. In 1628, Harvey published his work in a publication called *On the Movement of the Heart and Blood in Animals*. As you might imagine, at first many people did not believe Harvey's teachings. The nature of science sometimes dictates that good, new scientific knowledge takes time to become trusted.

A leech, sometimes used for bloodletting procedures. A leech can increase its body size considerably after feeding on a blood meal.



15th-century illustration of common bloodletting sites. The labelling is the original Latin. Bloodletting was sometimes done by making cuts and sometimes by the application of leeches.

Bloodletting was a common medical procedure that was based on Galen's theory of circulation. Bloodletting was a procedure whereby small cuts were made in order to drain blood from certain areas of the body. The thinking was that blood and other bodily 'humours' (fluids) needed to be in balance, and an illness was often attributed to these humours being out of balance. Bloodletting was believed to restore the healthy balance.

Exercises

4 Identify all the heart chambers, valves, and blood vessels involved in one complete circuit of blood (only blood vessels immediately entering or exiting the heart need to be named). Name these in the order the blood passes through them, starting with the right atrium.

5 Before birth, a human foetus has a hole between the right atrium and left atrium. Work out how that changes the blood flow within the foetal circulation, and why foetal circulation has evolved such a pattern.

6 What causes heart valves to open and close?

NATURE OF SCIENCE

Risks associated with scientific research: Florey and Chain's tests on the safety of penicillin would not be compliant with current protocol on testing.

The world that we live in is literally infested with viruses and bacteria. Only a very, very small percentage of these are pathogenic to human beings; in fact, the vast majority of bacteria are very useful.

6.3 Defence against infectious disease

Understandings

- The skin and mucous membranes form a primary defence against pathogens that cause infectious disease.
- Cuts in the skin are sealed by blood clotting.
- Clotting factors are released from platelets.
- The cascade results in the rapid conversion of fibrinogen to fibrin by thrombin.
- Ingestion of pathogens by phagocytic white blood cells gives non-specific immunity to diseases.
- Production of antibodies by lymphocytes in response to particular antigens gives specific immunity.
- Antibiotics block processes that occur in prokaryotic cells but not in eukaryotic cells.
- Viruses lack a metabolism and cannot therefore be treated with antibiotics. Some strains of bacteria have evolved with genes that confer resistance to antibiotics, and some strains of bacteria have multiple resistance.

Applications and skills

- Application: Causes and consequences of blood clot formation in coronary arteries.
- Application: Florey and Chain's experiments to test penicillin on bacterial infections in mice.
- Application: Effects of HIV on the immune system and methods of transmission.

Guidance

- *Diagrams of skin are not required.*
- *Subgroups of phagocyte and lymphocyte are not required but students should be aware that some lymphocytes act as memory cells and can quickly reproduce to form a clone of plasma cells if a pathogen carrying a specific antigen is re-encountered.*
- *The effects of HIV on the immune system should be limited to a reduction in the number of active lymphocytes and a loss of the ability to produce antibodies, leading to the development of AIDS.*

Primary defence is to keep pathogens out

Our bodies are exposed to many disease-causing agents. Any living organism or virus that is capable of causing a disease is called a pathogen. Pathogens include viruses, bacteria, protozoa, fungi, and worms of various types. Yet exposure to the vast majority of pathogens does not result in a disease. Primarily, this is because we are too well defended for most pathogens to enter our bodies and, if any do manage to enter, we have often previously developed immunity to that pathogen. For some pathogens, such as bacteria, there are chemicals called antibiotics that can work against the living bacterial cells but do not affect our body cells. Let's explore more about this interesting and important topic.

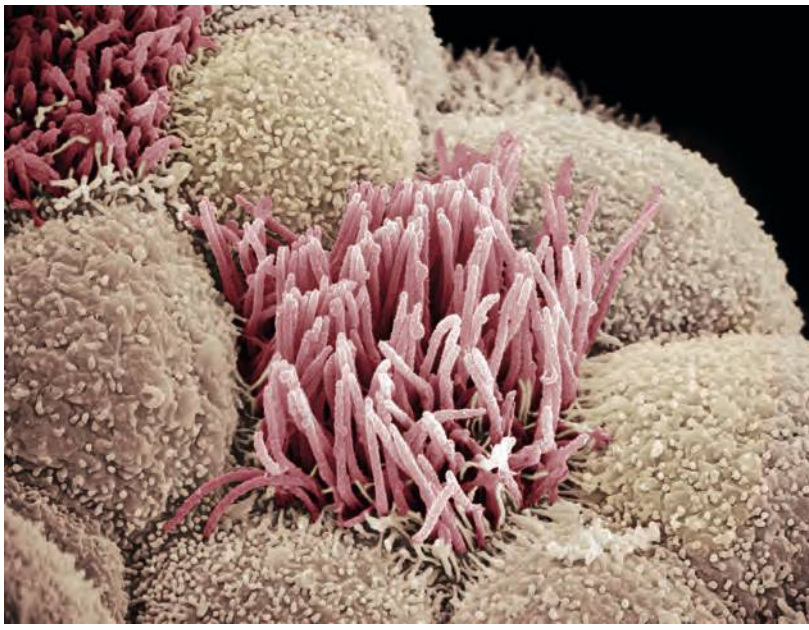
Skin and mucous membranes form a primary defence

The best way to stay healthy is to prevent pathogens from having the chance to cause disease. One way to do this is to try to stay away from sources of infection. This is why it is still common to isolate (or quarantine) people who have highly transmittable diseases. Obviously, it is not possible to isolate yourself from every possible source of infection. Therefore, the human body has some ingenious ways of making it difficult for pathogens to enter it and start an infection.

One of those ingenious ways is your skin. Think of your skin as having two primary layers. The underneath layer is called the dermis and is very much alive. It contains sweat glands, capillaries, sensory receptors, and dermal cells, which give structure and

strength to the skin. The layer on top of this is called the epidermis. This epidermal layer is constantly being replaced as the underlying dermal cells die and are moved upwards. This layer of mainly dead cells forms a good barrier against most pathogens because it is not truly alive. As long as our skin remains intact, we are protected from most pathogens that can enter living tissues. This is why it is important to clean and cover cuts and abrasions of the skin when they do occur.

Pathogens can enter the body at a few points that are not covered by skin. These entry points are lined with tissue cells that form a mucous membrane. Cells of mucous membranes produce and secrete a lining of sticky mucus. This mucus can trap incoming pathogens and so prevent them from reaching cells that they could infect. Some mucous membrane tissue is lined with cilia. Cilia are hair-like extensions capable of a wave-like movement. This movement moves trapped pathogens up and out of mucous-lined tissues such as your trachea. Table 6.4 shows some common areas that have a mucous membrane.



False-colour scanning electron micrograph (SEM) of the mucous membrane lining of the trachea. The large white cells are called goblet cells and they secrete mucus. Hair-like cilia (in pink) are also visible.

Table 6.4 The locations of mucous membranes

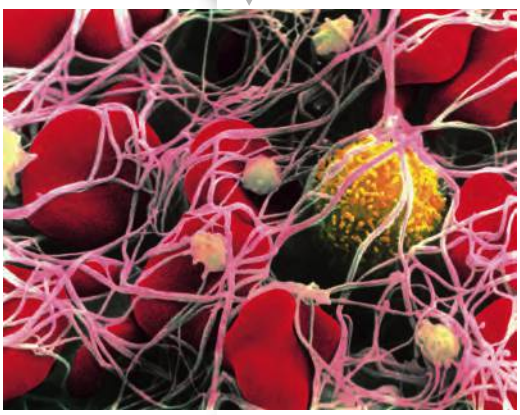
Area with a mucous membrane	What it is and does
Trachea	The tube that carries air to and from the lungs
Nasal passages	Tubes that allow air to enter the nose and then the trachea
Urethra	A tube that carries urine from the bladder to the outside
Vagina	The reproductive tract leading from the uterus to the outside

According to an article published by the National Institute of Health (NIH), bacteria outnumber their human hosts by about 10 to 1 cells. In a typical human adult, bacteria would account for about 2% of his or her body mass.

Blood clotting minimizes the chances of infection and blood loss

When small blood vessels like capillaries, arterioles, and venules are broken, blood escapes from the closed circulatory system. Often the damaged blood vessels are in the skin, and so pathogens then have a way to gain entry into the body. Our bodies have evolved a set of responses to create a clot that 'seals' the damaged blood vessels, so preventing excessive blood loss and helping prevent pathogens from entering the body.

Circulating in the blood plasma are a variety of molecules called plasma proteins. These proteins serve many purposes, including



This false-colour SEM shows that small platelets (shown in pale green) have triggered the formation of insoluble fibrin protein fibres. Trapped in the fibrin are several red blood cells, platelets, and one white blood cell (shown in yellow).

some that are involved in clotting. Two of the clotting proteins are prothrombin and fibrinogen. These two molecules are always present in blood plasma, but remain inactive until 'called to action' by events associated with bleeding. Also circulating in the bloodstream are cell fragments known as platelets. Platelets form in the bone marrow, along with red blood cells (erythrocytes) and white blood cells (leucocytes), but do not remain as entire cells. Instead, one very large cell breaks down into many fragments, and each of the fragments becomes a platelet. Platelets do not have a nucleus and they have a relatively short cellular life span, of about 8–10 days.

Let's consider what happens when a small blood vessel is damaged (see Figure 6.11). The damaged cells of the blood vessel release chemicals that stimulate platelets to adhere to the damaged area. The damaged tissue and platelets release chemicals called clotting factors that convert prothrombin into thrombin. Thrombin is an active enzyme that catalyses the conversion of soluble fibrinogen into the relatively insoluble fibrin. The appropriately named fibrin is a fibrous protein that forms a mesh-like network that helps to stabilize the platelet plug. More and more cellular debris becomes trapped in the fibrin mesh, and soon a stable clot has formed, preventing both further blood loss and the entry of pathogens.

Haemophilia is an inherited blood-clotting disorder. Haemophiliacs lack the ability to produce one of the chemicals needed for normal clotting.

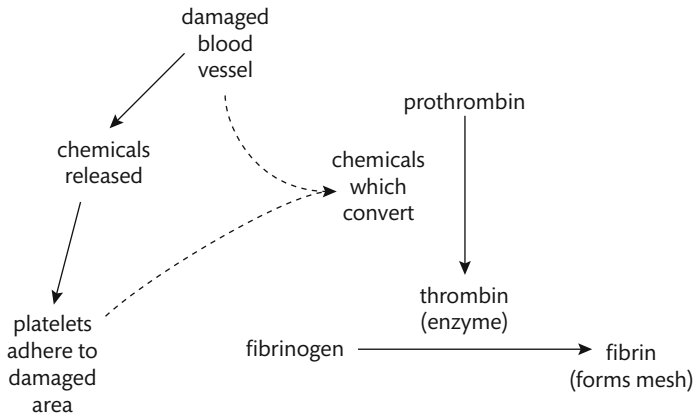


Figure 6.11 Flowchart of the blood-clotting sequence. The sequence starts with a damaged blood vessel, and leads to a meshwork of fibrin that traps blood cells to form a clot. The image on page 285 shows blood cells trapped in fibres of fibrin.

When pathogens get past skin and mucous membranes

Travel between far-reaching areas of our world has increased tremendously in the last century. With that increase in travel has come an associated increase in the rate of spread of global disease.

When a pathogen, such as a pathogenic bacterial species, does enter the body, a series of events begins known as the immune response. If this is a first encounter with a particular pathogen, the response is known as a primary immune response. If it is a second (or third, etc.) encounter, the response is known as a secondary immune response. A primary immune response takes at least a week or more to be successful, and thus it is common to experience the symptoms associated with a disease while the immune system is working to reduce and finally eliminate the pathogen. A secondary immune response is both quicker and more intense, and thus symptoms are rarely experienced. The ability to accomplish a secondary immune response for a particular antigen is actually what we call being 'immune' to a disease.

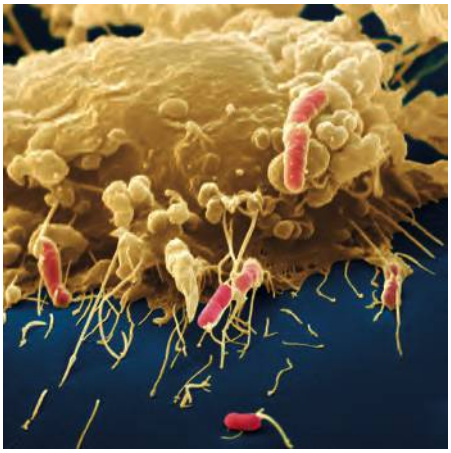
Role of phagocytic white blood cells

White blood cells (leucocytes) are the cells in our bloodstream that help us fight off pathogens that enter our bodies, and also provide us with immunity for the many pathogens that we encounter more than once. One type of leucocyte that is involved

very early on in the process of fighting off a pathogen is called a macrophage. Macrophages are large leucocytes that are able to change their cellular shape to surround an invading cell through the process of phagocytosis. Because macrophages can easily change their shape, they are able to squeeze their way in and out of small blood vessels. Therefore, it is not unusual for a macrophage to first encounter an invading cell outside the bloodstream.

When a macrophage meets a cell, it can recognize whether that cell is a natural part of the body and therefore 'self', or not part of the body and therefore 'not-self'. This recognition is based on the protein molecules that make up part of the surface of all cells and viruses. If the collection of proteins the macrophage encounters on a cell is determined to be 'self', then the cell is left alone. If the determination is 'not-self', the macrophage engulfs the cell by phagocytosis. Phagocytes typically contain many lysosome organelles, in order to digest chemically whatever has been engulfed. This type of response by the body is called non-specific, because the identity of the specific pathogen has not been determined, just the fact that it is something that is 'not-self' and therefore should be removed.

The role of macrophages in determining self versus not-self cells is called non-specific immunity, even though no real immunity is gained by the action of the macrophages.

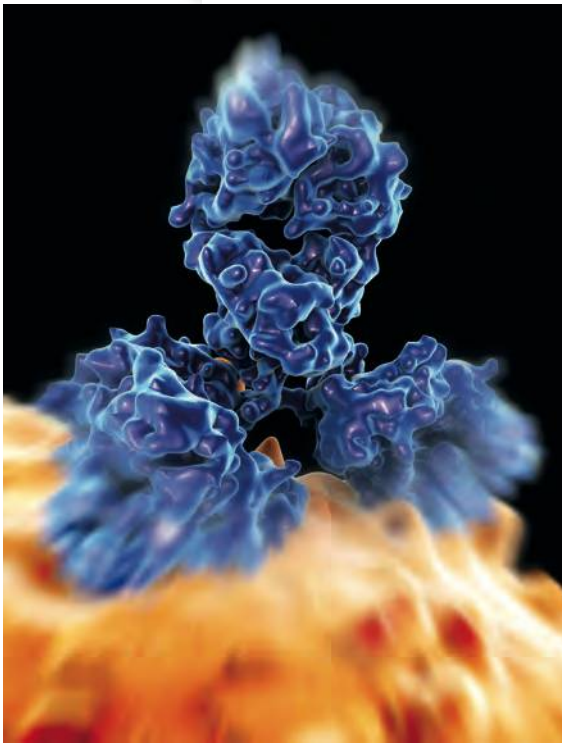


False-colour SEM showing a macrophage (the large yellow cell) engulfing *Escherichia coli* bacteria (the small pink rods).

Antibodies produced by lymphocytes lead to specific immunity

Antibodies are protein molecules that are produced by the body in response to a specific type of pathogen. In other words, if you had a measles infection, you would produce one type of antibody, and if you contract a virus that gives you influenza (flu), you would produce another type of antibody. Each type of antibody is different because each type has been produced in response to a different pathogen. Each pathogen is made up of either cells with cell membranes or, in the case of a virus, a protein coat called a capsid. The cellular invaders, such as bacteria, have proteins that are embedded in their outer surface. In the language of the immune system, these foreign proteins are called antigens. You have just learned that 'not-self' proteins trigger an immune response. All of these 'not-self' proteins are antigens.

Each antibody is a protein that is Y-shaped. At the end of each of the forks of the Y is a binding site. The binding site is where an antibody attaches itself to an antigen. Because the antigen is a protein on the surface of a pathogen (such as a bacterium), the antibody thus becomes attached to the pathogen (see the artwork on the right).

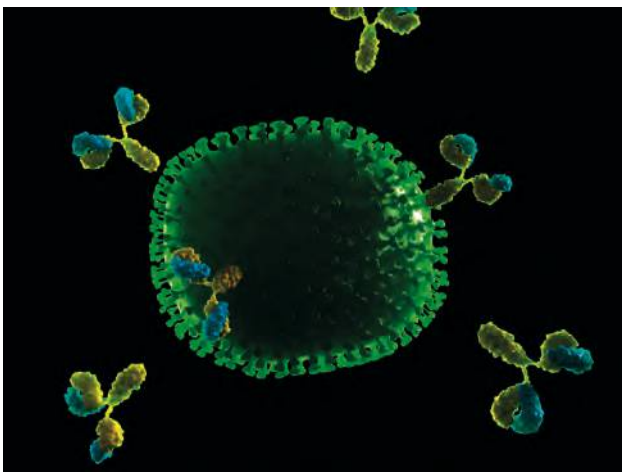


Computer artwork showing an antibody attaching to a cell surface. One way antibodies function is to attach to and thus 'mark' a cell for destruction by certain types of leucocytes.

The leucocytes that produce antibodies are a type of cell called plasma cells. Each of us has many different types of antibody-producing plasma cells and, as a general rule, each type of plasma cell can produce only one type of antibody. The problem is, each cell only produces a relatively small number of antibodies in comparison with the massive infection that may be present in the body. However, our continually evolving immune response has a way of producing many of the same type of plasma cells when they are needed. Here are the steps of a typical primary immune response.

1. A specific antigen type is identified (e.g. a particular cold virus).
2. A specific plasma cell is identified that can produce an antibody that will bind to the antigen (e.g. the proteins of the capsid coat of the cold virus).
3. The specific plasma cell type clones itself (divides repeatedly by mitosis) to increase rapidly the numbers of that type of plasma cell.
4. The newly formed 'army' of specific plasma cells begins antibody production.
5. The newly released antibodies circulate in the bloodstream and eventually find their antigen match (e.g. the proteins of the virus capsid).
6. Using various mechanisms, the antibodies help eliminate the pathogen.
7. Some of the cloned antibody-producing plasma cells remain in the bloodstream and provide immunity against a second infection by the same pathogen. These long-lived cells are called memory cells.
8. Memory plasma cells of this type respond quickly if the same antigen is encountered again (a secondary immune response).

Vaccines are weakened or non-pathogenic forms of pathogens that cause a primary immune response within your body. This leads to the production of the same memory lymphocytes as the actual disease does. Thus, following a vaccination, if you do encounter the actual pathogen, the memory cells will initiate a very quick secondary immune response. In most instances, the secondary immune response is so quick that symptoms associated with the pathogen do not have time to develop.



Artwork showing antibodies binding to a flu virus. Each antibody is uniquely designed to fit an antigen. This is part of your specific immunity, because of the specificity of the molecules involved in the 'match'.

What is HIV and how does it affect the human immune system?

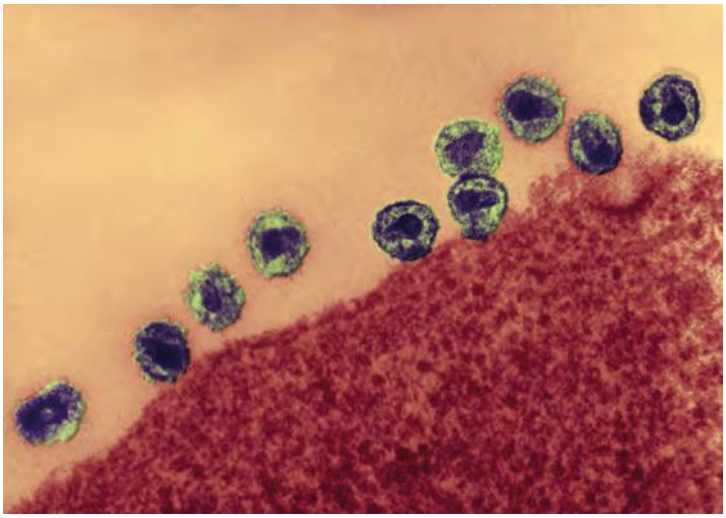
HIV is the abbreviation for a virus called human immunodeficiency virus. Just like any virus, HIV is very specific about which organisms and which cell types in an organism it infects. Unfortunately, the infected (host) cells in humans is one of the

key lymphocyte cell types involved in the immune responses just described. A person infected with HIV will eventually experience a severe drop in his or her lymphocyte population, and will lose the ability to produce adequate antibodies. It typically takes many years after the initial infection by HIV before an infected person loses his or her specific immune response capability, but when it does happen the resulting immune disease is called AIDS or acquired immune deficiency syndrome.

When the symptoms of AIDS do begin, the infected person can no longer fight off pathogens as he or she could before, and a multitude of infections of various types begins. It is one or more of these secondary infections that most often takes the life of someone with AIDS. At the time of publication of this text, no effective treatment has been found to cure someone with an HIV infection. However, a variety of treatments have been found that are prolonging the time period between infection and the onset of symptoms of AIDS.

How is HIV transmitted?

The two most common ways that HIV is spread from person to person is by having unprotected sex with an infected person, and by using a hypodermic needle that has previously been used by someone who is HIV-positive (HIV^+). In addition, it is possible for an HIV^+ mother to infect her child during pregnancy, labour, delivery, or breastfeeding. In some countries, receiving a blood transfusion can spread HIV, but that is no longer a risk in countries where blood and blood products are routinely tested for contamination. Some medical treatments, such as injections for treating haemophilia, have been known to spread HIV when the injection was purified from human blood. In many areas of the world, these products are now produced by genetically engineered bacteria and have no risk of transmitting HIV.



A false-colour TEM of HIV (small round objects) infecting a leucocyte.

TOK

Do scientific researchers have a responsibility to communicate and collaborate freely with each other? Sometimes a competitive environment, striving to be the first to discover something, can get in the way of productive collaboration. An example was the limited collaboration between competing USA and French research teams in the early days of research on the pathogen that we now know as HIV.

The use of antibiotics to combat bacterial infections

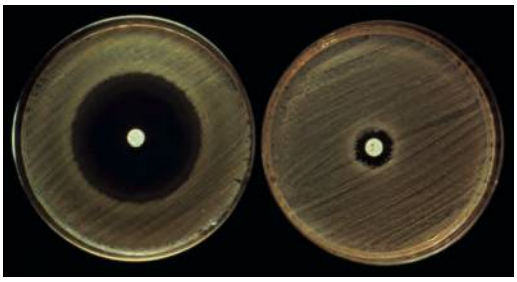
Bacteria are prokaryotic cells. Humans and other animals are composed of eukaryotic cells. There are major structural and biochemical differences between prokaryotic and eukaryotic cells. For example, protein synthesis is similar in both types of cell, but not exactly the same. Also, bacteria have a cell wall, a structure not characteristic of eukaryotic animal cells. Antibiotics are chemicals that take advantage of the differences

between prokaryotic and eukaryotic cells, and selectively block some of the biochemistry needed by bacteria while having no effect on human or animal cells. There are many categories of antibiotics, depending on the biochemical pathway that is being targeted. One type of antibiotic may selectively block protein synthesis in bacteria, but have no effect on our cells' ability to manufacture proteins. Another type may inhibit the production of a new cell wall by bacteria, thus blocking their ability to grow and divide.

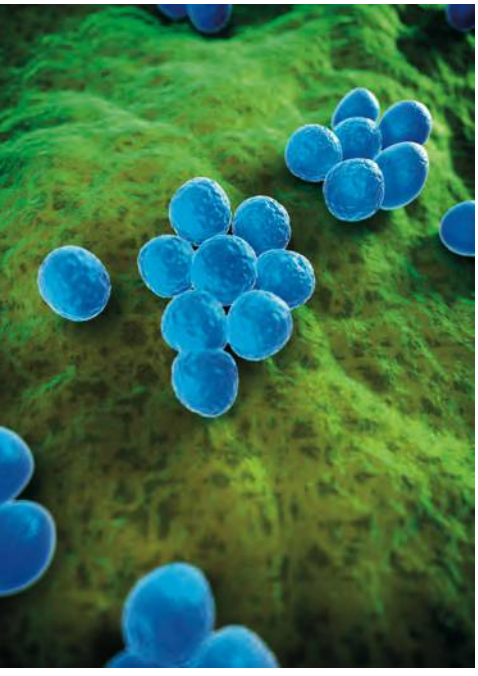
This also explains why antibiotics have no effect on viruses. Viruses make use of our own body cells' metabolism to create new viruses. Any chemical that could inhibit this would also be damaging to our own body cells. Thus antibiotics are chemicals with the ability to damage or kill prokaryotic cells, but not damage eukaryotic cells or their metabolism; because a virus has no metabolism of its own, antibiotics are not prescribed for any disease of viral origin.

NATURE OF SCIENCE

Alexander Fleming made the initial discovery of penicillin in 1928. However, Fleming became frustrated by his inability to isolate the chemical from the fungus that produced the antibiotic, and moved on to other work. About a decade later, Ernst Chain and Howard Florey picked up on Fleming's work and isolated a small amount of the penicillin compound. They injected eight mice with a deadly bacterial species and four of these mice were also injected with the newly isolated penicillin. The four mice that were not injected with penicillin all died within a day. The four mice that were injected with penicillin all lived for several days. Small-scale studies such as this would in fact have little credibility by the standards used today to judge the validity of experimental work.



At the centre of each Petri dish is a tablet of penicillin. As you can see, growth of the strain of bacteria on the left is greatly inhibited by the penicillin that is diffusing outwards from the pellet. The strain of bacteria on the right is a strain that has developed a resistance to penicillin and its growth is not nearly as inhibited.



Computer artwork showing MRSA bacteria (small blue spheres).

An unsolved dilemma: bacterial resistance to antibiotics

Remember that any one antibiotic is a specific chemical that selectively targets some aspect of prokaryotic cell biochemistry that is different from eukaryotes. Bacteria show genetic variation just like all other living organisms on Earth. Because bacterial population numbers can be incredibly large, and because bacteria can reproduce very quickly, the mathematical odds that within a bacterial population a genetic variant exists that is not affected by any one antibiotic is quite possible. That one (or a few) variant can then reproduce and repopulate a colony in a very short period of time with bacteria that are all resistant to the antibiotic. The surviving resistant bacteria would then be a new strain of bacteria.

The long-term use and overuse of antibiotics has now led to many pathogenic species of bacteria that have strains that are resistant to nearly all of the antibiotics in existence today.

Some strains of bacteria are even resistant to multiple antibiotics. *Staphylococcus aureus* is a bacterium that can be pathogenic, resulting in what many call a 'staph infection'. Some strains of *S. aureus* are referred to as MRSA (pronounced 'mersa'): these are strains of *S. aureus* that have developed a resistance to many types of antibiotics. MRSA infections are very difficult to treat and are becoming more and more frequent.

Exercises

7 Why are some pathogenic viruses potentially lethal (e.g. HIV, *Ebola*), while others result in only fairly mild and temporary symptoms?

8 Distinguish between non-specific and specific immune responses.

9 What is a virus doing when it is not infecting a host cell?

10 In the very early years of research on the disease that we now know as AIDS, government funding for research was close to non-existent. Other than the fact that it was a fairly 'new' disease, can you think of one or more reasons why funding was so low?

6.4 Gas exchange

NATURE OF SCIENCE

Obtain evidence for theories: epidemiological studies have contributed to our understanding of the causes of lung cancer.

Understandings:

- Ventilation maintains concentration gradients of oxygen and carbon dioxide between air in alveoli and blood flowing in adjacent capillaries.
- Type I pneumocytes are extremely thin alveolar cells that are adapted to carry out gas exchange.
- Type II pneumocytes secrete a solution containing surfactant that creates a moist surface inside the alveoli to prevent the sides of the alveolus adhering to each other by reducing surface tension.
- Air is carried to the lungs in the trachea and bronchi, and then to the alveoli in bronchioles.
- Muscle contractions cause the pressure changes inside the thorax that force air in and out of the lungs to ventilate them.
- Different muscles are required for inspiration and expiration because muscles only do work when they contract.

Applications and skills:

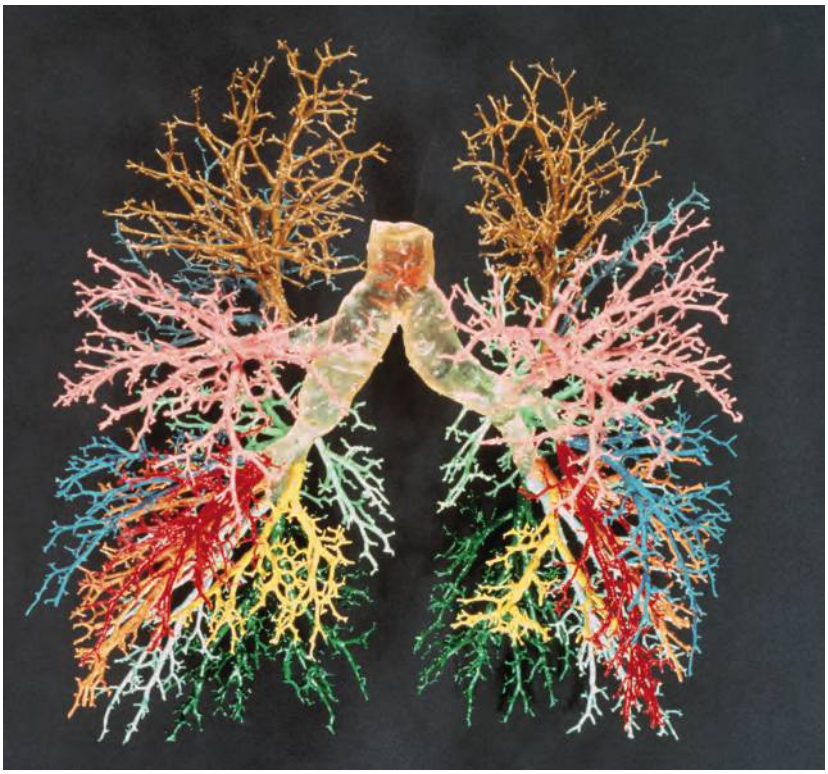
- Application: Causes and consequences of lung cancer.
- Application: Causes and consequences of emphysema.
- Application: External and internal intercostal muscles, and diaphragm and abdominal muscles, as examples of antagonistic muscle action.
- Skill: Monitoring of ventilation in humans at rest and after mild and vigorous exercise.

Guidance

- *Ventilation can either be monitored by simple observation and simple apparatus, or by data logging with a spirometer or chest belt and pressure meter. Ventilation rate and tidal volume should be measured, but the terms vital capacity and residual volume are not expected.*
- *Students should be able to draw a diagram to show the structure of an alveolus and an adjacent capillary.*

Overview of the respiratory system

Our lungs act in concert with our heart and blood vessels to ensure that body cells are well supplied with oxygen and are able to give up carbon dioxide. Most people



A resin cast image of airways in the lungs. The trachea divides into the right and left primary bronchi. Each primary bronchus continues to divide multiple times, leading to smaller and smaller bronchioles. You can see why the entire structure is sometimes called the 'bronchiole tree'.

never seriously consider why we need oxygen, but everyone knows that we do. The process that requires oxygen (and gives off carbon dioxide) is aerobic cell respiration. In brief, this is a biochemical pathway in which the chemical bonds within a glucose molecule are broken down sequentially to release energy. Much of this energy is then stored as molecules of adenosine triphosphate (ATP). In aerobic organisms, the process requires oxygen molecules, and each of the six carbons of a glucose molecule is given off as a carbon dioxide molecule.

Throughout our lives we continuously repeat the process of filling our lungs with air and then expelling that air. This is called ventilation. Even though the air we breathe is inside our lungs for only a short period of time, it is long enough for diffusion of gases to occur. Within the lungs are a multitude of small spherical air sacs called alveoli. Oxygen in the alveoli typically diffuses into the bloodstream, and carbon dioxide from the bloodstream typically diffuses into the alveoli. Each breath in and out maintains the concentration gradients that encourage diffusion of oxygen into and carbon dioxide out of the nearby capillary beds that are adjacent to the many alveoli making up the bulk of lung tissue.

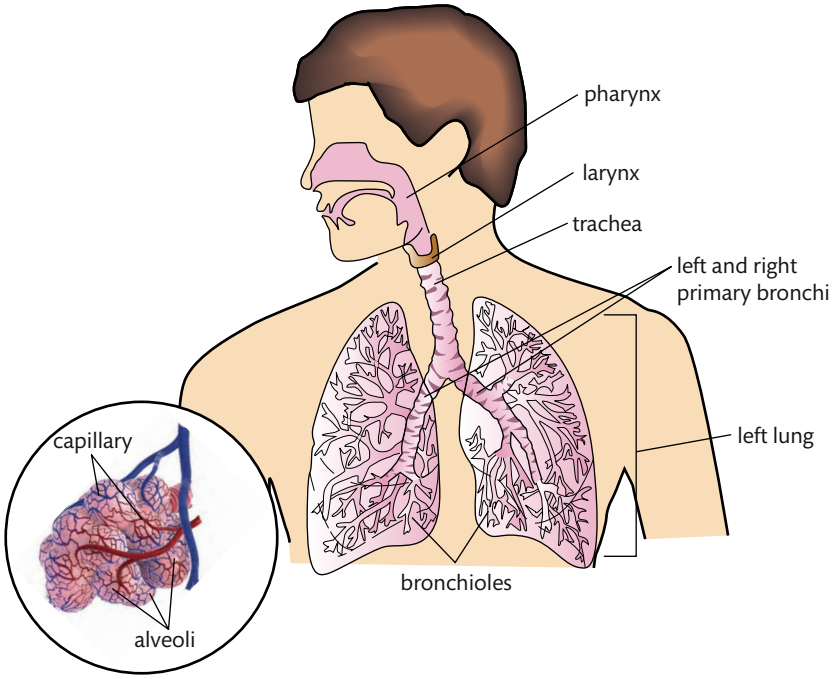
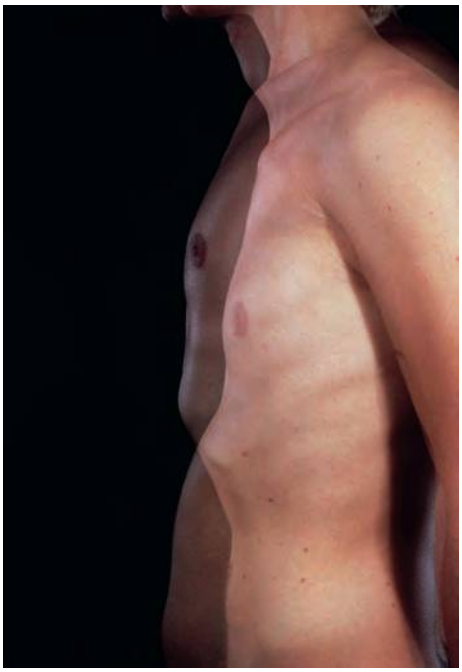


Figure 6.12 Air can enter the trachea from either the mouth or nasal passages. The inhaled air passes through the larynx (the voicebox with vocal cords) and then down the trachea. The trachea branches many times into multiple bronchioles. Finally the air reaches the small air sacs surrounded by rich capillary beds.

The mechanism of ventilation



A double-exposure photograph showing the position of the chest during inspiration and expiration. Inspiration is occurring when the chest/rib cage is in the raised position.

We breathe in and out continuously all our lives. Each time we take a breath, a fairly complex series of events occurs that we do not even think about as it is happening. The tissue that makes up our lungs is passive and not muscular, therefore the lungs themselves are incapable of purposeful movement. However, there are muscles surrounding the lungs, including the diaphragm, muscles of the abdomen, and the external and internal intercostal muscles (which surround your ribs).

The mechanism of breathing is based on the inverse relationship between pressure and volume (see Figure 6.13). Put simply, an increase in volume will lead to a decrease in pressure, and vice versa. Whatever pressure does, volume will do the opposite. Your lungs are located within your thoracic cavity (or thorax). The thoracic cavity is closed to the outside air. Your lungs have only one opening to the outside air, and that is through your trachea (via your mouth and nasal passages). Thus we need to consider the two environments that affect each other: one is the closed environment of the thorax, and the other is the internal environment of the lungs.

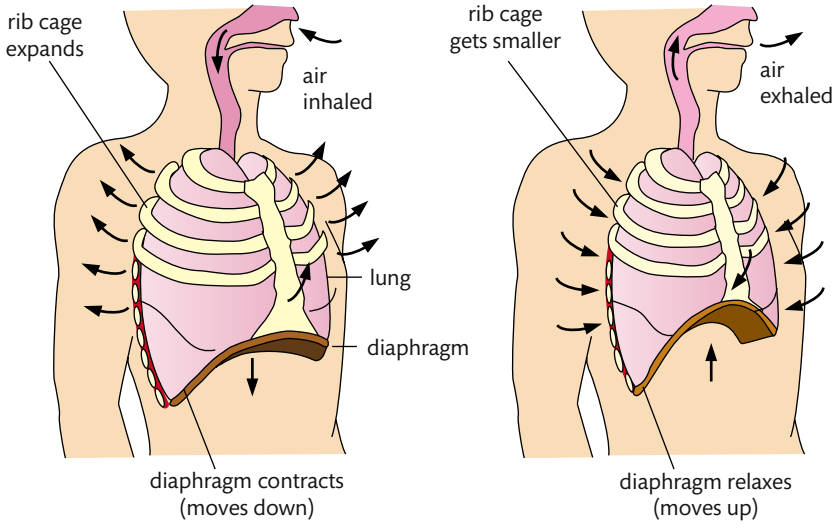


Figure 6.13 The mechanisms for inspiration and expiration (ventilation).

CHALLENGE YOURSELF

3 Create a list of steps that trace a single erythrocyte that begins in the capillary bed adjacent to an alveolus. Name the major blood vessels and heart chambers that the erythrocyte goes through until it returns to another capillary bed in the lungs. Hint: You will need to take the cell through the remaining pulmonary circuit, into a systemic circuit starting with the aorta, and then eventually back through the first portion of another pulmonary circuit.

Actions that lead to an inspiration (breathing in)

1 The diaphragm contracts, and at the same time the external intercostal muscles and one set of abdominal muscles help to raise the rib cage. Collectively, these actions increase the volume of the thoracic cavity.

CHALLENGE YOURSELF

4 List the five steps (in order) necessary for expiration, with the following as your starting point.
- The diaphragm relaxes and the internal intercostal muscles and a second set of abdominal muscles help to lower the rib cage. Collectively, these actions decrease the volume of the thoracic cavity.

Notice that different muscles are necessary for an inspiration versus an expiration. For example, the intercostal muscles are the muscles that are found between the ribs. There are two antagonistic sets of these muscles: external intercostals, which are used when breathing in, and the internal intercostals, which are used when breathing out.

2 Because the thoracic cavity has increased its volume, the pressure inside the cavity decreases. This leads to less pressure 'pushing on' the passive lung tissue.

3 The lung tissue increases its volume because there is less pressure exerted on it.

4 This leads to a decrease in pressure inside the lungs, also known as a partial vacuum.

5 Air comes in through your open mouth or nasal passages to counter the partial vacuum within the lungs, and fills the alveoli.

These steps are reversed for an expiration (breathing out).

All the steps become more frequent and exaggerated when you are exercising and thus breathing deeply. For example, the abdominal muscles and intercostal muscles achieve a greater initial thoracic volume. This leads to deeper breathing and thus more air moving into the lungs.

Monitoring ventilation in humans at rest and after mild and vigorous exercise

Safety alerts: Many schools and the IB Animal Experimentation policy require parent permission forms to be signed before any type of investigation of the pupils themselves is performed. If so, this must be completed well before the investigation begins.

Note: This investigation is best done as a whole class project with shared data sets.

This lab reinforces the concepts associated with changes in homeostatic mechanisms in the human body. Ventilation is the rate of breathing and is typically given as breaths min^{-1}. An increase in exercise predictably results in a greater use of oxygen and release of carbon dioxide to/from muscle tissue associated with the exercise.

Question

What is the correlation between ventilation rate and duration of exercise?

Hypothesis

Ventilation rate will be positively correlated with the increasing duration of a chosen exercise.

Planning steps necessary before beginning

Determine a safe exercise that can be accomplished by everyone that is happy to be a test subject. Typical examples might be walking up a flight of stairs or jumping jacks. Next, determine the maximum time duration that is both reasonable and safe for the exercise you have chosen. Hint: try to make it easy to subdivide your total duration time.

Summary of procedures

1 Choosing human subjects for experimentation is difficult as it is often not possible to account for comparable subjects based on criteria such as gender, age, body mass index (BMI) similarities, health, current level of activity (sports), and genetic background. You will probably have to make test groups from a very limited population of test subjects (e.g. your classmates). Try to set at least some limited criteria for test subjects. Try to make three to five test groups with as many test subjects in each group as possible. Five groups of five in each group would be ideal, but perhaps not realistic.

2 You will need baseline ventilation data for each individual test subject. Use a timer and count the number of breaths for a 20-second time period for each test subject. Record this as raw data and be sure to keep track of the identity of each person and his or her 20-second ventilation rate. The test subject can count his or her own breaths with someone else acting as a timer and recorder. An alternative is to use data logging hardware and software that is designed to measure ventilation rate and perhaps tidal volume (the volume of air in a single breath).

3 Individually, have each test subject do one, and only one, of the exercise durations you predetermined. Very soon after each subject has finished, take a 20-second count of his or her number of breaths and record that data, again making sure to keep track of who it is and the duration of his or her exercise. If the number of test subjects is very low, you may have to use one or more subjects for more than one exercise duration. If this is the case, make sure to allow as much recovery time between tests as possible.

Data-processing possibilities

- For each test subject, calculate a ventilation rate, expressed in breaths min^{-1}, for both the baseline and after-exercise raw data (the 20-second ventilation counts).
- For each test subject, calculate a percentage increase of ventilation rate, showing the increase after exercise compared with the baseline rate.
- Calculate the mean percentage increase for each group. Example: calculate the mean percentage increase for all the test subjects who did jumping jacks for 90 seconds.
- If your data set included at least five test subjects for each exercise duration, calculate the standard deviation of each of the means from the previous step.

Data-presentation possibilities

- Design and create a data table showing all the relevant raw data. Test subject numbers can be assigned instead of using names.
- Design and create a data table showing all the relevant processed data.
- Design and create a graph with exercise durations on the *x*-axis (with appropriate units) and mean percentage increases (% unit) on the *y*-axis.
- If the data set appears to be reasonably linear on your graph, draw a single best-fit line representing the overall data pattern.
- Add standard deviation error bars to each mean point plotted on your graph, and add a note to your graph that the error bars indicate standard deviation.

Gas exchange occurs in alveoli

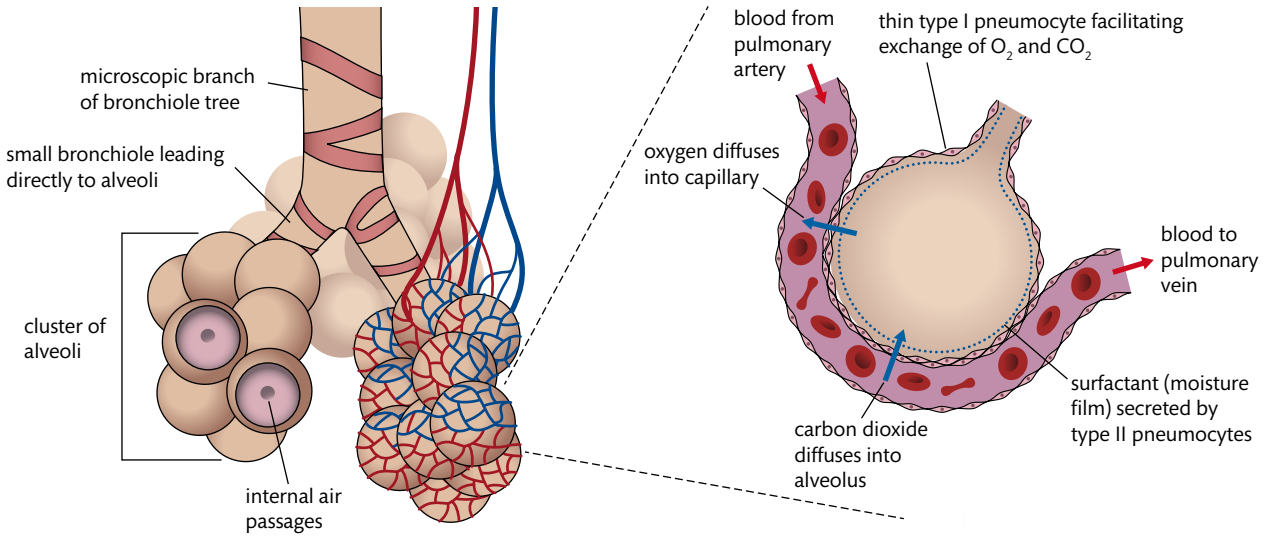


Figure 6.14 Microscopic view of a small area inside a human lung. Each cluster of alveoli is surrounded by a capillary bed for efficient gas exchange. The inset shows a sectioned drawing of a single alveolus and the structures that make gas exchange efficient.

When you take in air through your mouth or nasal passages:

- the air first enters your trachea
- then your right and left primary bronchi
- then smaller and smaller branches of the bronchi
- then very small branches called bronchioles
- then, finally, the air enters the small air sacs in the lungs called alveoli.

Alveoli in the lungs are found as clusters at the ends of the smallest bronchioles. In appearance they are very similar to a bunch of grapes. There are approximately 300 million alveoli in each of your lungs. Each cluster of alveoli has one or more surrounding capillary bed(s).

The blood entering these capillary beds comes from the right ventricle via the pulmonary arteries. As you will recall, blood within the pulmonary arteries is relatively low in oxygen and high in carbon dioxide. While this blood is in the capillary bed surrounding a cluster of alveoli, oxygen diffuses from the air in each alveolus through the membranes, which is only through two cells. The first of these is the single cell making up the structure of the alveolus, and the second is the single cell making up the wall of the capillary. Carbon dioxide diffuses in the opposite direction through the same two cells. As long as a person continues breathing, and refreshing the gases within the alveoli, the concentration gradients of these two gases will ensure diffusion of each gas in the direction that the body needs for healthy gas exchange.

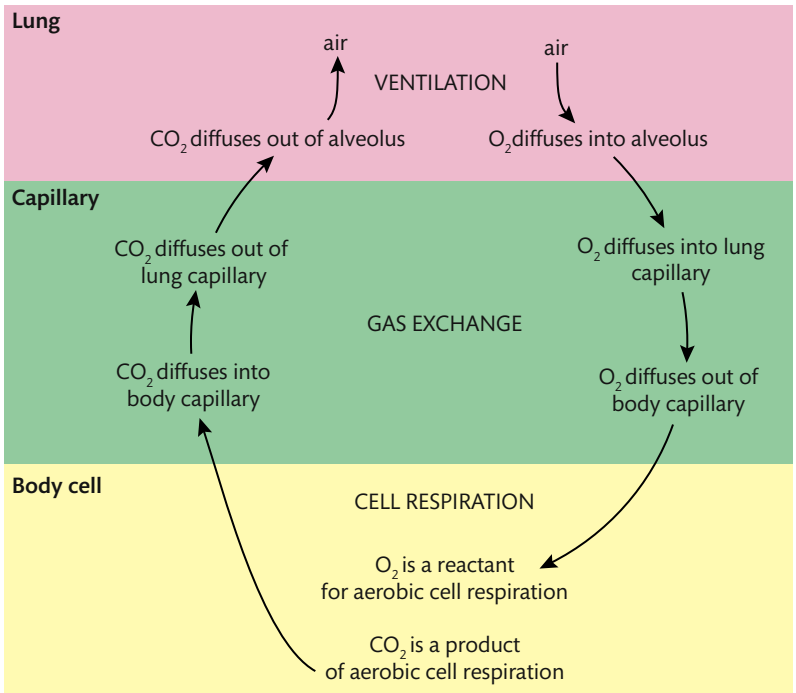


Figure 6.15 Relationship between ventilation, gas exchange and cell respiration.

Alveoli are composed of specialized cells called pneumocytes

An alveolus is an evolutionary marvel designed for efficient gas exchange. As mentioned above, one of the design features of an alveolus is that it is composed of a single layer of cells, to facilitate oxygen and carbon dioxide diffusion. This single cell layer is composed of two different types of cells called pneumocytes.

Type I pneumocytes

This type of alveolar cell is very thin but has a very large membrane surface area, making it well designed for diffusion. If damaged, these cells are incapable of mitosis for replacement.

Type II pneumocytes

This type of alveolar cell is cuboidal in shape and thus has relatively little membrane surface area. These cells produce and secrete a solution that acts as a surfactant. This reduces the surface tension of the moist inner surface of alveoli, and prevents the sides of the alveoli from sticking to each other. Type II pneumocytes are capable of mitosis for replacement of both types of alveolar cells if they are damaged.

Causes and consequences of emphysema

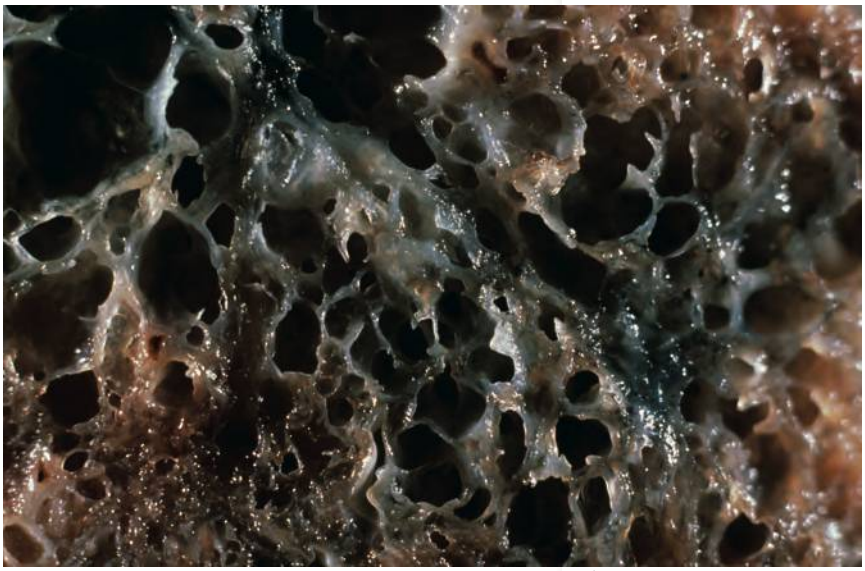
Emphysema is a disease whereby the alveoli in the lungs are progressively destroyed. The leading cause of emphysema is smoking. Emphysema is one of the diseases collectively known by the acronym COPD (chronic obstructive pulmonary disease). Emphysema is a chronic, slowly progressive disease that turns healthy alveoli into large, irregularly shaped structures with gaping holes. This reduces the surface area for gas exchange, and so less oxygen reaches the bloodstream. This explains the symptom described as 'shortness of breath'. At first, shortness of breath only occurs when the afflicted person does strenuous activity, but over time the inability to get sufficient gas exchange becomes constant.

Although long-term tobacco smoking is the leading cause of emphysema, there are other causes, including long-term exposure to the following:

- marijuana smoke
- fumes from manufacturing plants
- coal dust
- air pollution.

There is no cure for emphysema, but the progression of the disease can be slowed drastically with the cessation of smoking or exposure to other risk factors. To prevent emphysema, it is common sense not to even begin smoking, and to wear a protective mask when working around dust or chemical fumes.

The diagnosis of emphysema is often delayed because the symptoms develop slowly. People often associate the symptoms of emphysema with natural ageing, and people can initially find ways to compensate for their breathing problems.



Light microscope photograph of a section of lung taken from a diseased patient with emphysema. Notice the large gaping holes where healthy alveoli once were.

A better understanding of the causes of emphysema and lung cancer has led to massive campaigns to educate people about the dangers of smoking. In areas of the world where information concerning the dangers of smoking have been regularly and widely circulated, the percentage of people who smoke has declined.

Causes and consequences of lung cancer

Lung cancer is a cancerous growth that begins in the lungs. It is a cancer that is prone to spreading, a process called metastasizing. The brain, bones, liver, and adrenal glands are likely targets for lung cancer that has metastasized. The cancerous growth in the lungs takes over areas of healthy tissue areas that once provided a combination of bronchioles and alveoli. The larger the growth, the more the lung tissue becomes dysfunctional. Lung cancer can also result in internal bleeding in the lungs.

Lung cancer is caused by one or more carcinogen (a substance that is known to cause cancer) that enters the lung tissue and mutates cells into a cancerous growth. Sometimes the body is able to eliminate the early cancerous growth, but not always. More often than not, the carcinogen enters the lungs in cigarette smoke, although other fumes and substances have been known to be the source of the carcinogen.

The best treatment of lung cancer is achieved when the disease is diagnosed early in its progression. Lung cancer has a very high mortality rate.

Asbestos, once commonly used in building insulation products, is another carcinogen that can result in lung cancer. Many companies specialize in the safe removal of asbestos insulation from older buildings.

Once a company has freely admitted that its product is a risk to a consumer's health, does that admission eliminate the liability of that company in situations where the product does lead to poor health? This is the dilemma of current tobacco companies and the people that are addicted to their products.

TOK

Recent data supports a direct correlation between those countries and cultures that have shown a decrease in the number of people who smoke and a corresponding decrease in the incidence of lung cancer. Conversely, those areas of the world that are showing an increase in the number of people smoking are showing an increase in the incidence of lung cancer.

Exercises

11 Stopping smoking seems like such an easy, simple thing for people to do. Why do you think more people are not successful at stopping?

12 How are alveoli well adapted for efficient gas exchange?

13 Why are there two sets of muscles involved in ventilation (breathing)?

NATURE OF SCIENCE

Cooperation and collaboration between groups of scientists: biologists are contributing to research into memory and learning.

6.5 Neurones and synapses

Understandings:

- Neurones transmit electrical impulses.
- The myelination of nerve fibres allows for saltatory conduction.
- Neurones pump sodium and potassium ions across their membranes to generate a resting potential.
- An action potential consists of depolarization and repolarization of the neurone.
- Nerve impulses are action potentials propagated along the axons of neurones.
- Propagation of nerve impulses is the result of local currents that cause each successive part of the axon to reach the threshold potential.
- Synapses are junctions between neurones and between neurones and receptor or effector cells.
- When presynaptic neurones are depolarized they release a neurotransmitter into the synapse.
- A nerve impulse is only initiated if the threshold potential is reached.

Applications and skills:

- Application: Secretion and reabsorption of acetylcholine by neurones at synapses.
- Application: Blocking of synaptic transmission at cholinergic synapses in insects by binding of neonicotinoid pesticides to acetylcholine receptors.
- Skill: Analysis of oscilloscope traces showing resting potentials and action potentials.

Guidance

- *The details of structure of different types of neurones are not needed.*
- *Only chemical synapses are required, not electrical, and they can simply be referred to as synapses.*

The organization of the human nervous system

The brain and spinal cord comprise the central nervous system (CNS). These two structures receive sensory information from various receptors, and then interpret and process that sensory information. If a response is needed, some portion of the brain or spinal cord initiates a response that is called a motor response.

The cells that carry this information are called neurones. Sensory neurones bring information in to the CNS, and motor neurones carry response information to muscles.

Together, sensory neurones and motor neurones make up the peripheral nerves. A neurone is an individual cell that carries electrical impulses from one point in the body to another, and does so very quickly. When many individual neurones group together into a single structure, that structure is called a nerve. Think of a nerve as being like a

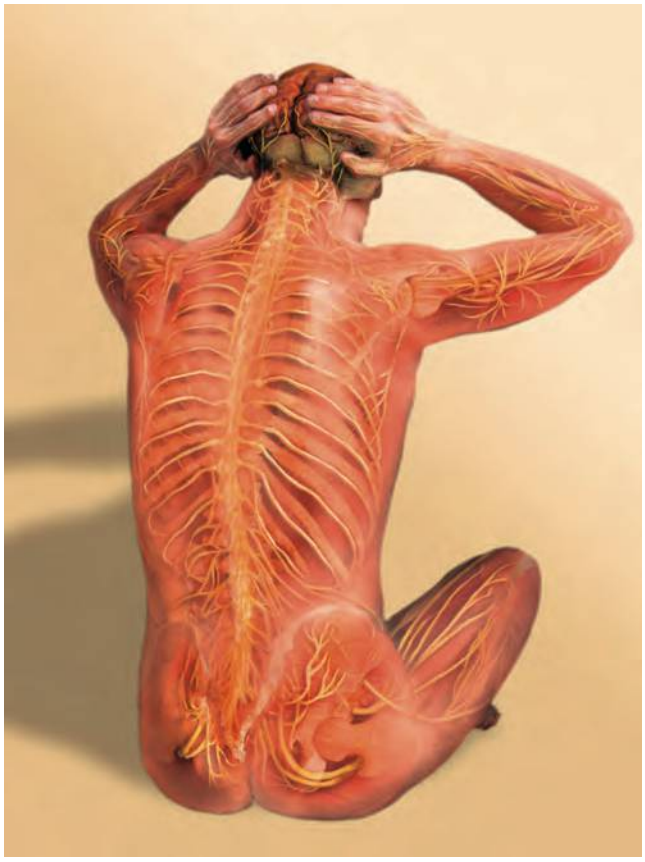
telephone cable: a protective sheath surrounding many individual wires. Each wire within that cable is like a neurone. The connection between the CNS and your body is made by two sets of nerves.

- Spinal nerves emerge directly from the spinal cord. They are mixed nerves, as some of the neurones within them are sensory and some are motor. There are 31 pairs of spinal nerves.
- Cranial nerves emerge from an area of the brain known as the brainstem. One well known example is the optic nerve pair, which carries visual information from the retina of the eyes to the brain. There are 12 pairs of cranial nerves.

Neurones

The cells that have been evolutionarily designed to transmit electrical impulses are called neurones. Neurones can be unusually long. In the human body, there are neurones that extend from the lower portion of the spinal cord all the way to the big toe: single cells that extend a distance of about 1 m! Of course, not all neurones are that long; in fact, some neurones are quite short.

Blue whales have some neurones that are approximately 25 m in length.



The central nervous system (CNS) consists of the brain and spinal cord. The peripheral nervous system (PNS) is made up of the nerves and branches that enter and leave the spinal cord and brainstem

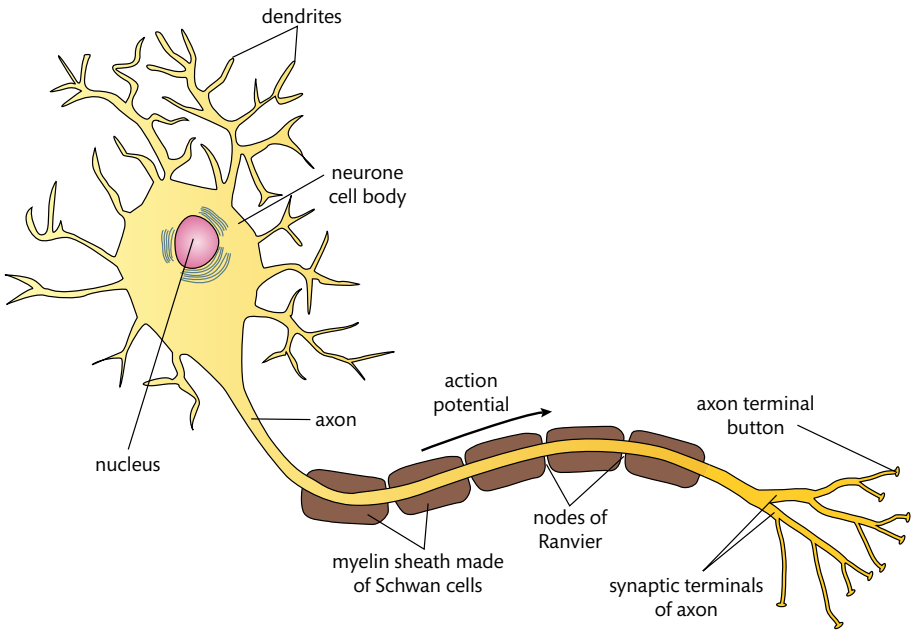
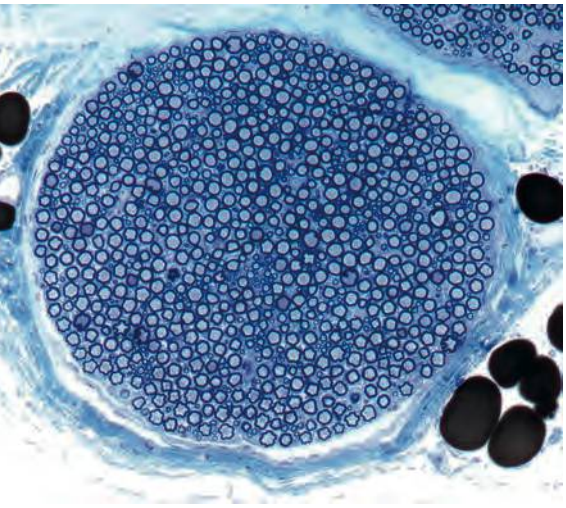


Figure 6.16 The structure of an individual neurone. The function of the myelin sheath and nodes of Ranvier are described on pages 302–303.

The three main subparts of a single neurone are its dendrites, cell body and axon. At the end of the axon are synaptic terminal buttons, which release chemicals called neurotransmitters that continue the impulse chemically to the next neurone(s) or possibly a muscle. An impulse is always carried from the dendrite end of a neurone along the membrane of the cell body down the axon, and results in a release of a neurotransmitter. The impulse does not travel in the opposite direction because neurotransmitter molecules cannot be released from the dendrite end of neurones, and the 'message' would simply stop at that point.



Light microscope photograph of a section of a nerve. The very large circle is the entire nerve, and each small circle within it is one of the axons of a neurone contained within that nerve.

What is a nerve impulse?

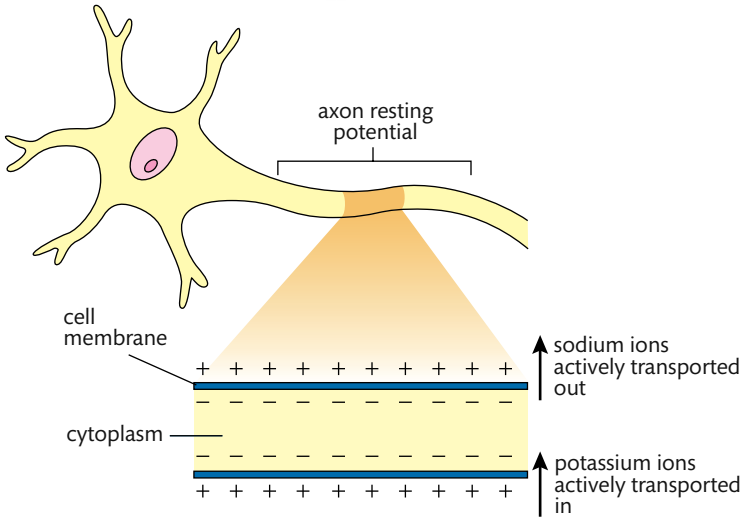
People often equate a nerve impulse to electricity. In some ways this is accurate, as a nerve impulse can be measured in the same way as electricity. For example, an action potential (or impulse) has a voltage, although the typical unit for this voltage is millivolts. In other ways, however, electricity and action potentials are very different. True electricity is a flow of electrons down a conductor; this is not the nature of an action potential. Let's look at what a nerve impulse actually is.

The term 'nerve impulse' is very misleading because a nerve does not carry an impulse; the individual neurones within the nerve are each capable of carrying the impulse. As axons of neurones are typically quite long, it is convenient to think of the conductor of a neurone impulse as the axon. The axons of neurones in some organisms (including humans) that have a very highly developed nervous system, have surrounding membranous structures collectively called the myelin sheath. The myelin sheath greatly increases the rate at which an action potential passes down an axon. In order to study the nature of an action potential, it is best to study an axon that does not have a myelin sheath, otherwise known as a non-myelinated neurone.

Figure 6.17 A neurone axon at resting potential. Think of the axon as a three-dimensional 'tube', and thus the ion movements shown are occurring all around the tube.

Resting potential: not currently sending an impulse

Let's look first at what an axon of a neurone is like when it is not sending an impulse. The time period during which an area of a neurone is ready to send an action potential, but is not actually sending one, is called the resting potential, and this area of the neurone is said to be polarized. The resting potential is created by the active transport of sodium ions (Na^+) and potassium ions (K^+) in two different directions. The vast majority of the sodium ions are actively transported out of the axon cell into the intercellular fluid, and the majority of the potassium ions are transported into the cytoplasm. This active transport of sodium and potassium in opposite directions is an active transport mechanism called the sodium–potassium (Na/K) pump. The Na/K pump works by transporting three sodium ions 'out' for every two potassium ions 'in'. In addition, there are negatively charged organic ions permanently located in the cytoplasm of the



axon. The net result of the position of the charged ions leads to a net positive charge outside the axon membrane (positive in relation to the inside) and a net negative charge inside the axon membrane (see Figure 6.17).

Depolarization: sending an impulse

An action potential is often described as a self-propagating wave of ion movements in and out of the neurone membrane. The movement of the ions is not along the length of the axon, but instead consists of ions diffusing from outside the axon to the inside, and from inside the axon to the outside. The resting potential requires active transport (the Na/K pump) to set up a concentration gradient of both sodium and potassium ions. As sodium ions are actively transported to the outside of the membrane, they diffuse in when a channel opens. This diffusion of sodium ions is the 'impulse' or action potential, and results in the inside of the axon becoming temporarily positive in relation to the outside. It is a nearly instantaneous event that occurs in one area of an axon, and is also called a depolarization. This depolarized area of the axon then initiates the next area of the axon to open up the channels for sodium, and thus the action potential continues down the axon. This is the self-propagating part of an action potential; once you start an impulse at the dendrite end of a neurone, that action potential will self-propagate to the axon end of the cell, where the synaptic terminals are located.

Each action potential must reach a minimum threshold in order to be self-propagated. This begins at the first receptor neurone that began the chain of events. A receptor neurone is a neurone that is modified to begin the sequence of events by transducing (converting) a physical stimulus of some kind into the first action potential. For example, some of the cells that make up the retina of your eyes are receptor cells. Each type of retinal cell has a minimum physical stimulus magnitude that is required in order to begin the impulse. For some retinal cells this is a minimum intensity of light. If that minimum intensity is not reached, no action potential begins. If the minimum is reached, an action potential is initiated and begins to self-propagate. There is no such thing as a strong impulse or a weak impulse: if the minimum threshold for that type of receptor is reached, an impulse begins. When a nerve impulse is being self-propagated along a neurone, that is happening because each successive area of the neurone membrane has reached its threshold and is causing the next area of the membrane to also reach its threshold.

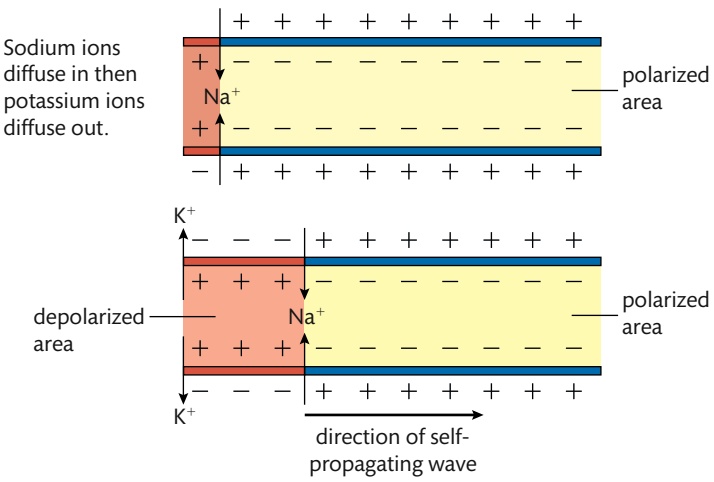


Figure 6.18 A neurone axon during and shortly after a depolarization.

Typically we are not aware of single impulses that reach our brain. If we sense a small amount of pressure on some area of our skin, it is because a few pressure receptors in that area have reached their threshold. If we feel a greater pressure, it is because the pressure has caused even more receptors in that area to reach their minimum threshold.

Nerve impulses are action potentials propagated along the axons of neurones.

Repolarization: return to the resting potential

Neurones do not send just one action potential; one neurone may send dozens of action potentials in a very short period of time. When one area of an axon has opened a channel to allow sodium ions to diffuse in, that area cannot send another action potential until ions have been restored to the positions characteristic of the resting potential. Diffusion cannot do this, thus active transport is required to pump ions to their resting potential positions.

If you recall from earlier, a depolarization is when sodium ions diffuse through the axon membrane from outside to inside. This means that, for a very short period of time, both sodium ions and potassium ions are inside together (this is why the inside of the membrane becomes positive relative to the outside). You may also recall that the active transport mechanism that resulted in the resting potential positions of sodium and potassium was the Na/K pump. This pump only works by moving sodium in one direction and potassium in the other direction across the membrane. Thus, immediately following an action potential (depolarization), membrane proteins open to potassium ions and allow them to diffuse out of the axon. This is the first step of repolarization because it separates many of the sodium and potassium ions on different sides of the membrane. The problem is that these two ions are on the opposite side of the membrane in relation to where they need to be for the resting potential. The good news is that they are now in a position that allows the Na/K pump to once again begin actively transporting them across the membrane at the ratio characteristic of this pump (three sodium ions pumped out for every two potassium ions pumped in). This entire series of events, beginning with potassium ions diffusing out of the localized area of the membrane, is called repolarization. All of this is necessary for that local area of the membrane to be ready to send another impulse.

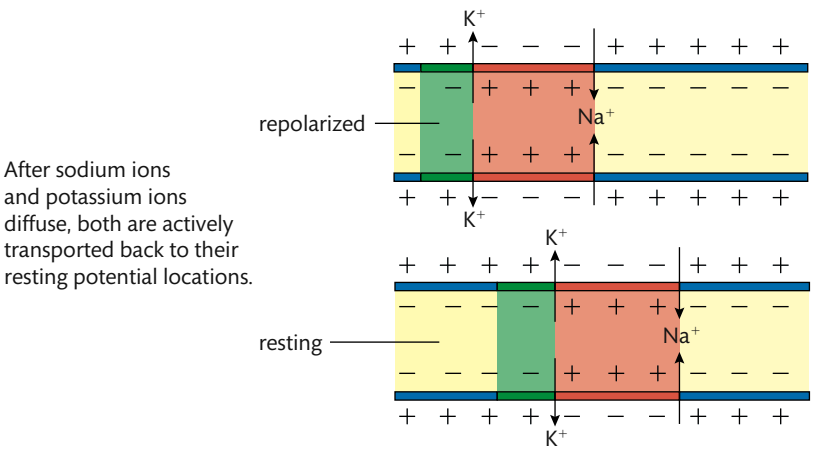


Figure 6.19 Return to the resting potential.

Saltatory conduction by neurones that have a myelin sheath

Many neurones of an organism with an advanced nervous system have axons with a myelin sheath; they are said to be myelinated. As an axon is like a long fibre, these axons are sometimes referred to as myelinated fibres. The myelin sheath is actually a series of cells, called Schwann cells, that have each wrapped themselves around the axon multiple times, creating multiple layers of the same cell membrane. The Schwann cells are spaced evenly along any one axon, with small gaps between them; these gaps are called nodes of Ranvier.

The term saltatory comes from the Latin word 'saltare', which means to hop or leap.

Saltatory conduction is the term used to describe the phenomenon whereby an action potential of myelinated axons skips from one node of Ranvier to the next as the impulse progresses along the axon towards the synaptic terminals. In other words, the action potential does not have to undergo the time-consuming and energy-expensive ion movements in the area of the membrane underneath the myelin material. The reason for this is that the myelin sheath acts as an insulator, preventing charge leakage through the membrane. The cytoplasm within the axon is electrically conductive, which allows the electrical potential to skip from one node of Ranvier to the next. The advantage of this is two-fold.

- The impulse travels much faster compared with an impulse in non-myelinated fibres, because the in/out ion movements characteristic of an impulse take time, and saltatory conduction allows areas of the membrane to be skipped. This is very important for the efficient neural processing characteristic of organisms with a high functioning nervous system.
- Less energy in the form of ATP is expended for the transmission of impulses, as the only locations where the Na/K pump needs to re-establish resting potentials is at the nodes of Ranvier.

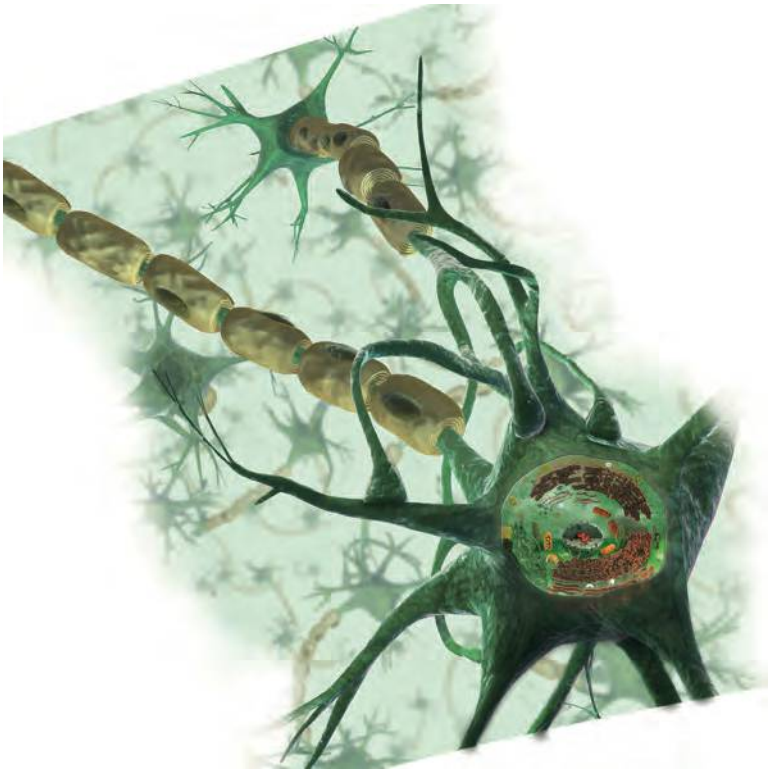
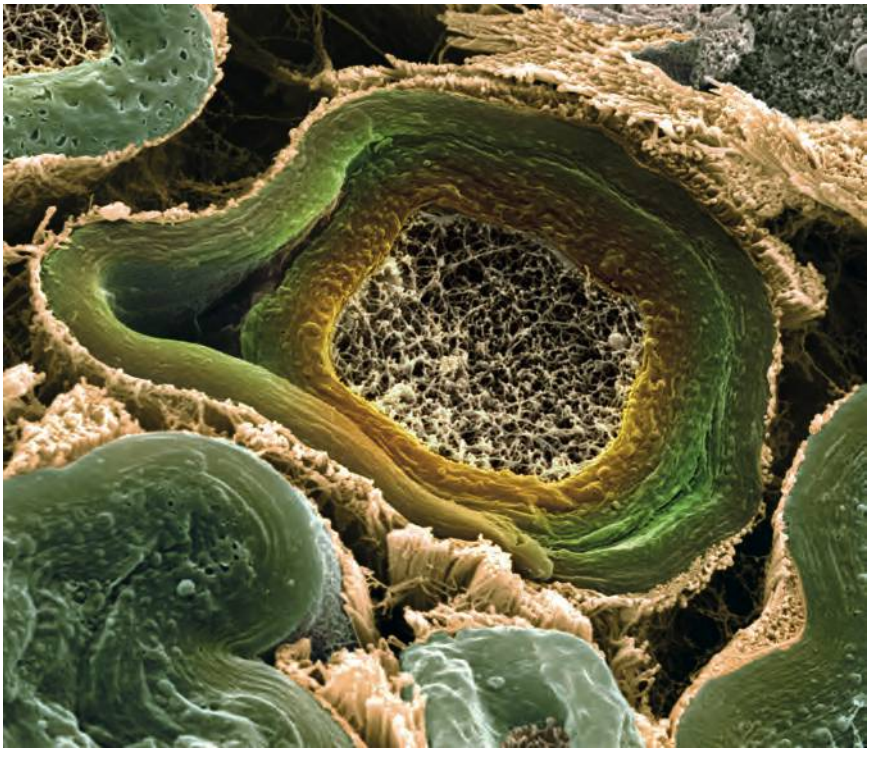
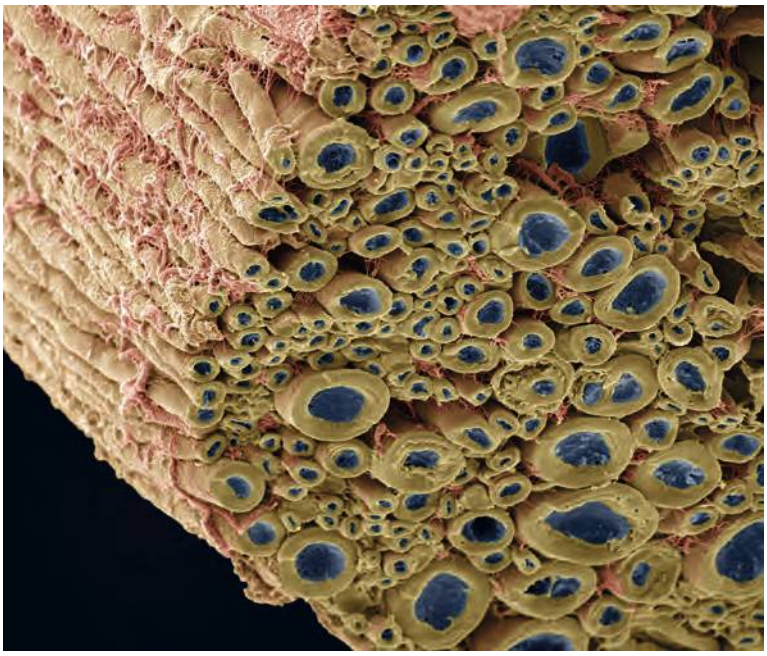


Illustration showing neurones with myelinated axons and nodes of Ranvier.



A false-colour SEM of a sectioned neurone with a myelin sheath. The axon is the centre beige area, and the myelin sheath is the surrounding yellow and green area.



A false-colour SEM of a nerve (bundle of neurones) with myelin sheaths. The blue colour shows the axons, and the surrounding yellow is the myelin sheath of each axon.

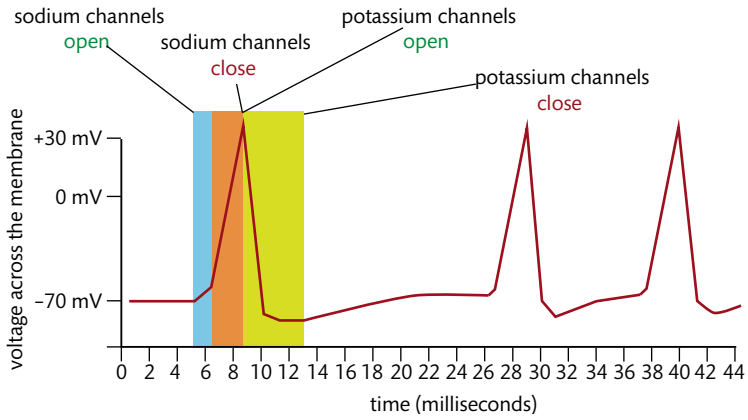


Figure 6.20 A graph showing the voltage changes across the membrane of an axon for three nerve impulses. Some of the important events are labelled on one of these impulses.

CHALLENGE YOURSELF

5 Use Figure 6.20, showing the change in voltage for a neurone sending impulses down its axon, to answer the following questions.

- **(a)** If each spike on this graph shows an impulse somewhere in the middle of an axon, what event must have just occurred in the area of the axon just preceding this one?
- **(b)** If the axon shown is myelinated, where along the axon did these voltage changes occur?
- **(c)** If this graph shows an impulse somewhere in the middle of an axon, and this is a myelinated fibre, what area of the axon will next undergo an action potential?
- **(d)** Where along the *x*-axis of the graph would the sodium–potassium pump be beginning to work to re-establish a resting potential?
- **(e)** What do you think would happen if discrete sensory information from a receptor was being received repeatedly at a rate faster than about 5 milliseconds apart?

Synapses: chemical communication between neurones

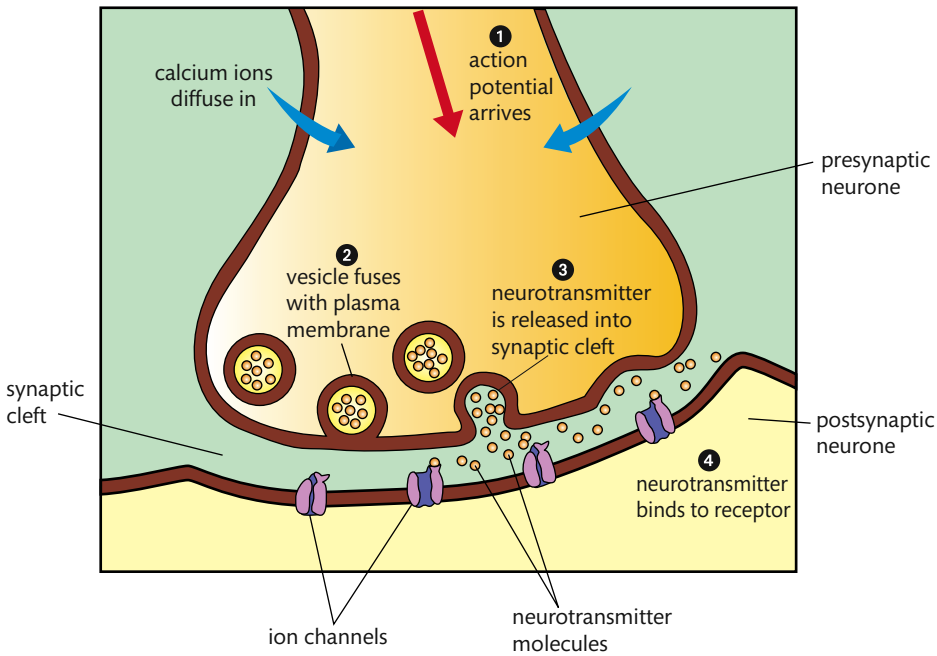
When one neurone communicates with another, the communication is chemical and occurs where two (or more) neurones adjoin each other in an area called a synapse. The two neurones always align with each other so that the axon's synaptic

terminals of one neurone adjoin the dendrites of another neurone. The chemical, called a neurotransmitter, is always released from the synaptic terminal buttons of the first neurone, and typically results in a continuation of the impulse when the neurotransmitter is received by the dendrites of the second neurone. The neurone that releases the neurotransmitter is called the presynaptic neurone, and the receiving neurone is called the postsynaptic neurone.

At the distal end of axons, as part of the synaptic terminals, are swollen membranous areas called terminal buttons. Within these terminal buttons are many small vesicles filled with the chemical neurotransmitter. There are many examples of neurotransmitters; a very common example in humans is acetylcholine.

When an action potential reaches the area of the terminal buttons, it initiates the following sequence of events (see Figure 6.21).

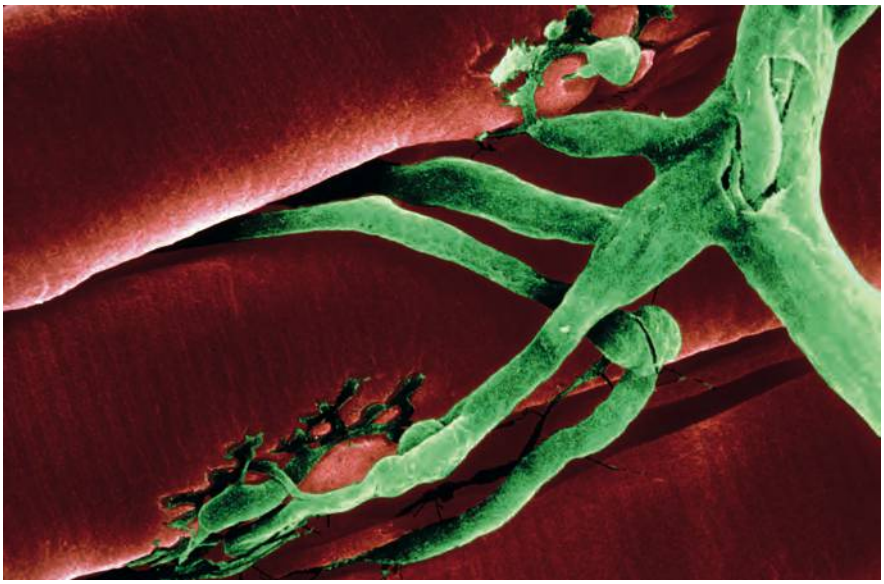
1. Action potential results in calcium ions (Ca^{2+}) diffusing into the terminal buttons.
2. Vesicles containing the neurotransmitter fuse with the plasma membrane and release the neurotransmitter.
3. The neurotransmitter diffuses across the synaptic gap (or cleft) from the presynaptic neurone to the postsynaptic neurone.
4. The neurotransmitter binds with a receptor protein on the postsynaptic neurone membrane.
5. This binding results in an ion channel opening and sodium ions diffusing in through this channel.
6. This initiates the action potential to begin moving down the postsynaptic neurone because it is now depolarized (the action potential is now self-propagating).
7. The neurotransmitter is degraded (broken into two or more fragments) by a specific enzyme(s) and neurotransmitter is released from the receptor protein.
8. The ion channel closes to sodium ions.
9. Neurotransmitter fragments diffuse back across the synaptic gap to be reassembled in the terminal buttons of the presynaptic neurone (often called reuptake).



Many mental disorders are associated with imbalances of certain neurotransmitters within the brain. There are approximately 50 different neurotransmitters that have been identified as active in the human brain. An imbalance of just one can result in conditions such as schizophrenia or severe depression. A large number of pharmaceuticals have been developed to treat these conditions based on our knowledge of how synapses and neurotransmitters work.

Synapses can also occur where a motor neurone adjoins muscle tissue. This type of synapse is called a motor end plate or neuromuscular junction. The mechanism for this type of synapse is almost the same as a neurone–neurone synapse, although the end result leads to the muscle undergoing a contraction. Another place for a synapse is between a receptor neurone (cell) of the nervous system and the first sensory neurone.

Figure 6.21 The mechanism of synaptic transmission.



Synapses can be between a receptor and first sensory neurone, or between two neurones, or between a motor neurone and muscle. This false-colour SEM shows a synapse between a neurone (green) and a muscle fibre (red).

Early studies of neonicotinoid pesticides suggested that they were relatively safe from an ecological viewpoint. More recent studies are showing some possible links to the 'colony collapse syndrome' being experienced by honeybee colonies. Each country must consider the mounting evidence, but chemicals in our environment have ways of crossing international borders through water, air, and many other means. If neonicotinoids are shown to cause damage to honeybee colonies, an international effort to curtail or stop their use will be necessary.

A new class of insecticides based on blocking synaptic transmission

Neonicotinoid insecticides are a relatively new class of insecticide that are chemically similar to nicotine. This type of insecticide works by binding to postsynaptic receptors that normally accept the neurotransmitter acetylcholine. When acetylcholine binds to the receptor protein, the result is the normal continuation of the action potential along the postsynaptic neurone. When neonicotinoid molecules bind to the same receptor proteins, the action potential is not propagated. In addition, the neonicotinoid molecules are not broken down by the enzyme acteylcholinesterase and thus the receptor becomes permanently blocked. This leads to a paralysis of the affected insect, and eventually death.

NATURE OF SCIENCE

The fields of psychology, chemistry, biology, and medicine all combine to contribute to our knowledge of memory and learning. One of the many complications for research on memory and learning is the sheer complexity of the human brain. Often, complex biological systems are best studied by using simpler 'models' that represent the more complex activity.

Biologists often use invertebrates that have a simpler nervous system compared with humans and other vertebrates. One interesting invertebrate is a sea snail called *Aplysia*. This marine snail can be stimulated to retract its siphon when it is touched, as part of its defence mechanism. The snail can learn from experience, and can keep its siphon protected for a longer period of time after being given a chance to learn. In addition, repeated touching of the siphon leads to a greater number of synapses between neurones in the very simple brain of *Aplysia*. This can be observed and documented because *Aplysia* has very few, but very large, neurones that can be easily seen. Use the hotlinks at the end of this section to see a video of *Aplysia* and this research.

Exercises

14 Explain the advantage that myelinated neurones have over non-myelinated neurones.

15 Individual neurones do not send action potentials with different 'strengths'. An action potential is either propagated (sent) or it is not. What is the term that describes the minimum electric potential necessary to propagate an impulse?

16 Arrange these events in the correct sequence to represent synaptic transmission.

(a) Binding of neurotransmitter to receptor protein on postsynaptic neurone.
(b) Enzyme degrades neurotransmitter.
(c) Ca^{2+} ions enter synaptic (terminal) buttons.
(d) Reuptake of neurotransmitter fragments.
(e) Neurotransmitter diffuses across synaptic gap.
(f) Na^{+} ions diffuse into postsynaptic neurone channels.

To learn more about *Aplysia*, go to the hotlinks site, search for the title or ISBN and click on Chapter 6: Section 6.5.

6.6 Hormones, homeostasis, and reproduction

Understandings:

- Insulin and glucagon are secreted by β and α cells in the pancreas, respectively, to control blood glucose concentration.
- Thyroxin is secreted by the thyroid gland to regulate the metabolic rate and help control body temperature.
- Leptin is secreted by cells in adipose tissue and acts on the hypothalamus of the brain to inhibit appetite.
- Melatonin is secreted by the pineal gland to control circadian rhythms.
- A gene on the Y chromosome causes embryonic gonads to develop as testes and secrete testosterone.
- Testosterone causes prenatal development of male genitalia and both sperm production and development of male secondary sexual characteristics during puberty.
- Oestrogen and progesterone cause prenatal development of female reproductive organs and female secondary sexual characteristics during puberty.
- The menstrual cycle is controlled by negative and positive feedback mechanisms involving ovarian and pituitary hormones.

Applications and skills:

- Application: Causes and treatment of Type I and Type II diabetes.
- Application: Testing of leptin on patients with clinical obesity and reasons for the failure to control the disease.
- Application: Causes of jet lag and use of melatonin to alleviate it.
- Application: The use in IVF of drugs to suspend the normal secretion of hormones, followed by the use of artificial doses of hormones to induce superovulation and establish a pregnancy.
- Application: William Harvey's investigation of sexual reproduction in deer.
- Skill: Annotate diagrams of the male and female reproductive system to show names of structures and their functions.

Guidance

- *The roles of FSH, LH, oestrogen, and progesterone in the menstrual cycle are expected.*
- *William Harvey failed to solve the mystery of sexual reproduction because effective microscopes were not available when he was working, so fusion of gametes and subsequent embryo development remained undiscovered.*

NATURE OF SCIENCE

Developments in scientific research follow improvements in apparatus: William Harvey was hampered in his observational research into reproduction by lack of equipment. The microscope was invented 17 years after his death.

Homeostasis

The human body typically stays within certain limits for many physiological variables. This is referred to as homeostasis. Here are some representative physiological variables:

- blood pH
- blood carbon dioxide concentration
- blood glucose concentration
- body temperature
- water balance within tissues.

Each of these variables has an expected value or set point that is considered to be normal for homeostasis. For example, you often hear that our internal body temperature is 37°C (98.6°F). However, there is an inevitable fluctuation around this exact temperature, depending on what a person has been doing, for example exercising or being out in very cold weather.

The physiological processes that bring a value back towards to a set point are called negative feedback mechanisms. Think of negative feedback control as working like a thermostat. The thermostat triggers one set of actions that is required when a value rises above its set point, and another set of actions when a value falls below its set point. Thus negative feedback functions to keep a value within the narrow range that is considered normal for homeostasis.

The nervous and endocrine systems work cooperatively in order to ensure homeostasis. Many of the homeostatic mechanisms initiated by your nervous system are under the control of your autonomic nervous system. The endocrine system consists of numerous glands that produce a wide variety of hormones. Each hormone is transported by the bloodstream from the gland where it is produced to the specific cell types in the body that are influenced by that particular hormone.

There are two main categories of glands. Exocrine glands are those that produce a secretion (enzyme, saliva, etc.) that is carried to a nearby, specific, location via a duct. Endocrine glands always produce one or more hormones, and these hormones are always secreted into the blood for distribution throughout the body.

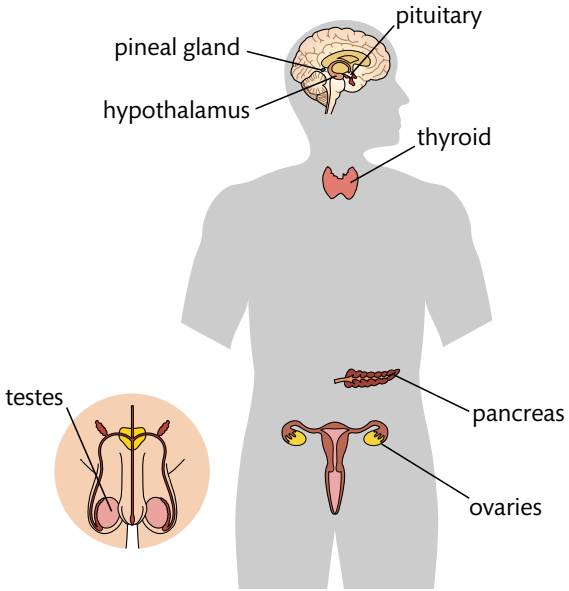


Figure 6.22 Some of the more common endocrine glands within the human body. Each of these glands produces one or more hormones that are secreted into the bloodstream and are carried to target tissues within the body. Target tissues are those cells that are influenced by any one hormone.

Selected hormones and their functions

Each hormone has a specific gland that produces and secretes the hormone into nearby capillary beds for distribution to body cells. Not all body cells are influenced by any one hormone: those cells that are influenced by a hormone are called the target

tissue(s) of the hormone. Some hormones (e.g. leptin) have very specific and limited target tissues, while others (e.g. insulin) have a broad range of target tissues.

Thyroxin

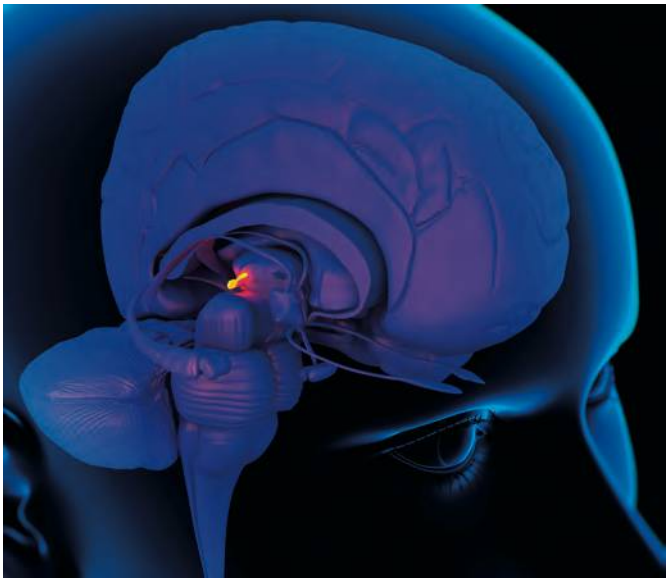
The gland that produces and secretes thyroxin is a 'butterfly'-shaped gland located in your neck called the thyroid gland. Thyroxin is created from an amino acid and iodine, and exists in two forms, one called T4 and the other called T3. The numbers indicate the number of iodine atoms within the structure. Both T3 and T4 enter the target cells (almost all cells in the body), where the T4 form is typically converted to the T3 form. The T3 form enters the nucleus of the cell and acts as a transcription regulator, leading to an increase in messenger (m)RNA and thus a resultant increase in proteins. Ultimately these proteins lead to an increase in the metabolism of the cell. Thus a cell under the influence of thyroxin will have a greater need for oxygen and other indicators of an increased metabolic rate. Someone who secretes too much thyroxin is said to have hyperthyroidism, and someone who secretes too little is said to have hypothryroidism. Both conditions can have serious symptoms.

In addition to increasing the metabolic rate, thyroxin helps to regulate internal body temperature. An increase in metabolic rate produces more heat from the increased chemical reactions that are occurring. Therefore an increase in thyroxin will lead to an increase in body temperature, and vice versa.

As mentioned, the synthesis of thyroxin requires iodine. People whose diets are deficient in iodine can develop hypothyroidism. Over time, with a deficiency of iodine, the thyroid gland tries to compensate by growing larger, and becomes markedly visible as it swells in size. An enlarged thyroid growth is called a goitre. Iodine deficiency has in fact become very rare in modern humans because most table salt has iodine added to it, thus it is sold as 'iodized salt'.

Leptin

Leptin is a hormone that is produced by adipose (fat) tissue in the body. The more fat stored in the body, the more leptin is produced and secreted into the bloodstream. Leptin's target cells are in the hypothalamus of the brainstem. Under ideal circumstances, leptin has the effect of lowering your appetite. Evolutionarily, the logic is simple: if someone has enough fat reserves, that person does not need to eat as much anymore. Unfortunately that simple logic doesn't always hold true, as evidenced by the very large incidence of obesity in modern society today. People who are obese are known to have a greater level of leptin circulating in their bloodstream. Researchers are working on why they appear to have become 'desensitized' to this high level of the appetite-controlling hormone. Some researchers have suggested that the function of leptin is related to increasing appetite when fat reserves are low, but not as an appetite suppressant when fat reserves are high.



The pineal gland highlighted in a sectioned view of the human brain. The right cerebrum and a portion of the brainstem has been removed in order to show the location of this small gland associated with sleep/wake cycles.

Melatonin

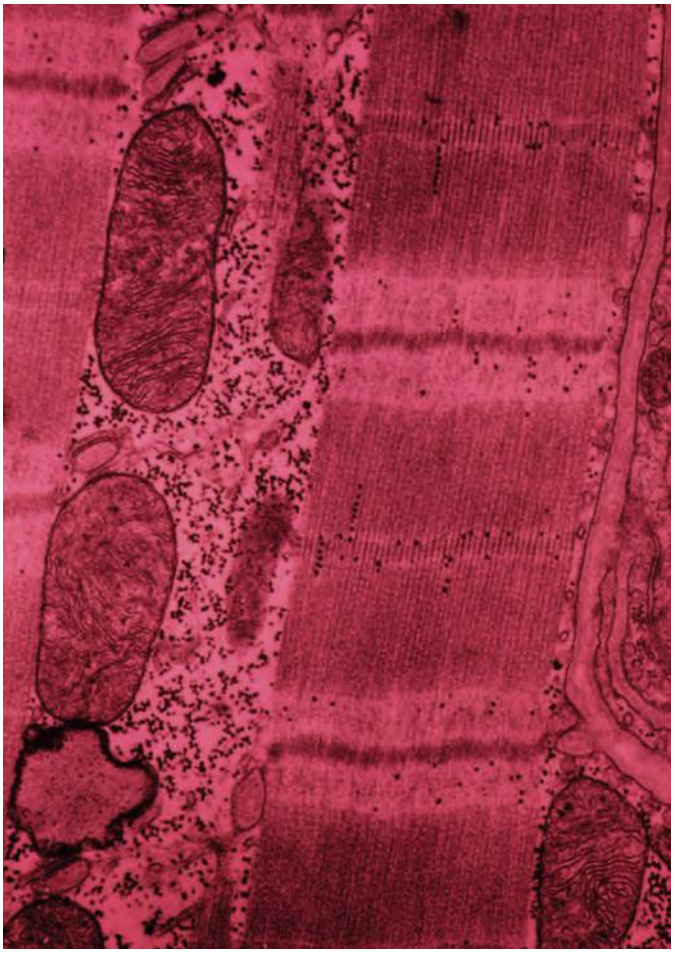
Deep within your brain is a very small gland called the pineal gland. Many animals use their pineal gland to help regulate their daily 24-hour cycle of activity, called the circadian rhythm. The hormone produced and secreted from the pineal gland is called melatonin. The pineal gland produces very little melatonin during the daytime, and is at peak production after dark, with maximum production occurring between 2 a.m. and 4 a.m. The natural circadian rhythm is altered when a person alters his or her period of exposure to light over a short period of time, especially when coupled

with a disruption of their normal sleep schedule. This is what is typically called 'jet lag', produced when a person travels through several time zones in a short period of time. Similar disorientation symptoms can be felt by people who work temporary night shifts or have other irregular time patterns of sleep versus being awake. Many people report a decline in the disorienting effects of jet lag by taking melatonin pills until their own circadian rhythm has naturally reset.

Insulin and glucagon help regulate glucose levels

Insulin and glucagon are hormones that are both produced and secreted by the pancreas. In addition, they are both involved in the regulation of blood glucose levels. Cells rely on glucose for the process of cell respiration. Cells never stop cell respiration and thus are constantly lowering the concentration of glucose in the blood. Many people eat three or more times a day, including foods containing glucose, or carbohydrates that are chemically digested to glucose. This glucose is absorbed into the bloodstream in the capillary beds of the villi of the small intestine, and thus increases the blood glucose level. So one factor that causes our blood glucose levels to fluctuate is simply that our blood does not receive constant levels of glucose. The increase and decrease in blood glucose levels goes on 24 hours a day, every day of your life. However, even though blood glucose is expected to fluctuate slightly above and below the homeostatic normal level, it must be maintained reasonably close to the body's set point for blood glucose level, and negative feedback mechanisms ensure this.

TEM of a cardiac muscle cell. Granules of glycogen can be seen as small black dots. Glucose is stored as glycogen in liver and muscle cells, and later can be reconverted back to glucose. Two mitochondria (ellipses) can be seen on the left.



In the intestinal villi, the glucose travels through a multitude of capillaries, small venules, and veins into the hepatic portal vein, which takes the blood to the liver. The glucose concentration in the hepatic portal vein varies depending on the time of your last meal and the glucose content of the food you ate. The hepatic portal vein is the only major blood vessel in the body in which blood levels fluctuate to a large degree. All other blood vessels receive blood after it has been processed by liver cells called hepatocytes. Hepatocytes are triggered into action by the two pancreatic hormones, insulin and glucagon. These two pancreatic hormones are antagonistic: they have opposite effects on blood glucose concentration.

What happens when blood glucose begins to rise above the set point?

In the pancreas there are cells known as β (beta) cells that produce the hormone insulin. Insulin is then secreted into the bloodstream and, because all body cells communicate chemically with blood, all cells are exposed to insulin. Insulin's effect on body cells is to open protein channels in their plasma membranes. These channels allow glucose to diffuse into the cell by the process known as facilitated diffusion.

There is another important effect attributed to insulin. When blood that is relatively high in glucose enters the liver by the hepatic portal vein, insulin stimulates the hepatocytes to take in the glucose (a monosaccharide) and convert it to glycogen (a polysaccharide). The glycogen is then stored as granules in the cytoplasm of the hepatocytes. The same effect occurs in muscles (see the TEM on the previous page).

The two effects of insulin both have the same ultimate result, which is to lower the glucose concentration in the blood or, to put it more simply, to reduce blood glucose.

What happens when blood glucose begins to fall below the set point?

The blood glucose level typically begins to drop below the set point when someone has not eaten for many hours or exercises vigorously for a long time. In either situation, the body needs to use the glycogen made and stored by the liver (and muscle cells). Under these circumstances, α (alpha) cells of the pancreas begin to produce and secrete the hormone glucagon. The glucagon circulates in the bloodstream and stimulates hydrolysis of the granules of glycogen stored in hepatocytes and muscle cells; the hydrolysis produces the monosaccharide glucose. This glucose then enters the bloodstream. The ultimate effect is to increase the glucose concentration in the blood or, to put it more simply, to increase blood glucose (see Figure 6.23).

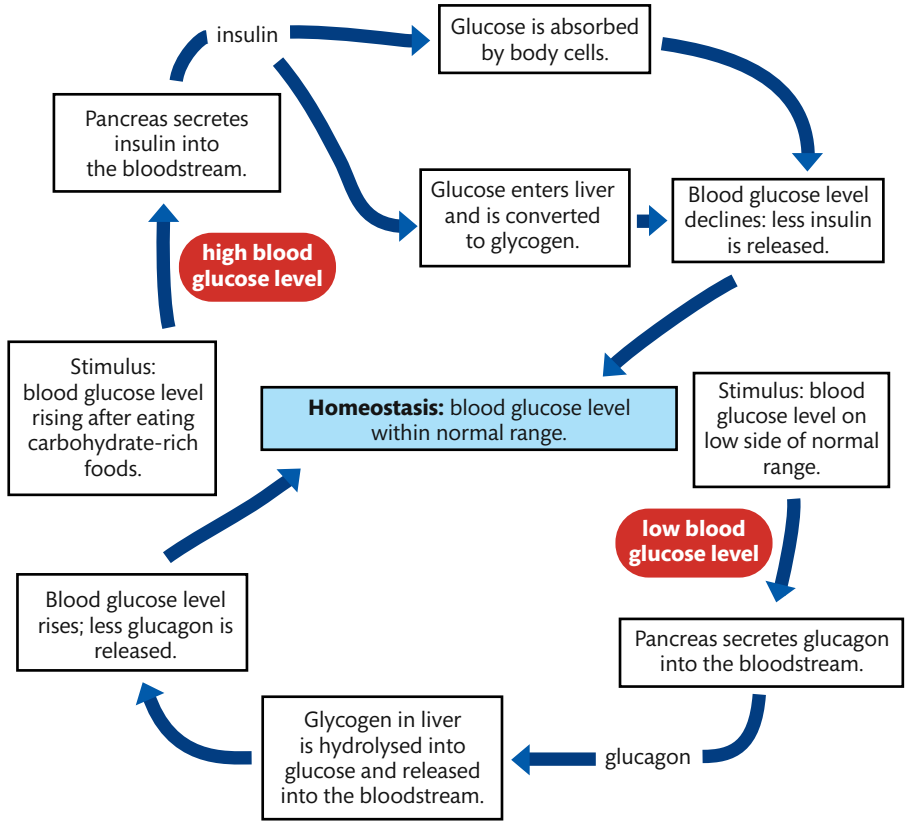


Figure 6.23 Negative feedback control of blood glucose level.

An endocrinologist is a physician who specializes in disorders associated with one or more hormones that are either under-produced (hyposecretion) or over-produced (hypersecretion). Hormone therapy is a branch of medicine that attempts to correct resulting disorders. Common examples are insulin for diabetes, melatonin for sleep disorders, and reproductive hormones following female menopause.

Diabetes

Diabetes is a disease characterized by hyperglycaemia (high blood glucose). Type I is typically caused when the β cells of the pancreas do not produce sufficient insulin; type II diabetes is caused by body cell receptors that do not respond properly to

insulin. You will recall that the hormone insulin should result in increased facilitated diffusion of glucose (through channels) into almost all body cells. This diffusion into body cells lowers the amount of glucose in the bloodstream. People who have untreated diabetes have sufficient glucose in their blood, but not in their body cells where it is needed.

Type I diabetes is controlled by the injection of insulin at appropriate times. Type II diabetes is controlled by diet. Uncontrolled diabetes of either type can lead to many serious effects, including:

- damage to the retina, leading to blindness
- kidney failure
- nerve damage
- increased risk of cardiovascular disease
- poor wound healing (and possibly gangrene, thus making amputation necessary).

Type I diabetes is an autoimmune disease. The body's own immune system attacks and destroys the β cells of the pancreas so that little or no insulin is produced by individuals with type I diabetes. Less than 10% of diabetics have this type of the disease. Type I diabetes most often develops in children or young adults, but can develop in people of any age.

The top three countries for the number of people with diabetes are: (1) China (more than 90 million); (2) India (more than 60 million); (3) USA (more than 23 million).

Type II diabetes is the result of body cells no longer responding to insulin as they once did. This is known as insulin resistance. Initially, the pancreas continues to produce a normal amount of insulin, but this level may decrease after a period of time. Type II diabetes is the most common form of diabetes; approximately 90% of diabetics have this type. Type II diabetes is often associated with genetic history, obesity, lack of exercise and advanced age, and is more common in certain ethnic groups.

Human reproduction

Despite all of the cultural 'trappings' that societies incorporate into the process of human reproduction, it is basically a male gamete (sperm) fertilizing a female gamete (egg or ovum). This cellular union ensures that half of the genetic makeup of the resulting zygote is derived from each parent. Thus, like all forms of sexual reproduction, reproduction in humans serves the bigger purpose of ensuring genetic variation in the species. In both sexes, hormones play a key role in both the development of sexual dimorphism (different body forms of males and females) and the regulation of sexual physiology.

For example, in males the hormone testosterone:

- determines the development of male genitalia during embryonic development
- ensures the development of secondary sex characteristics during puberty
- ensures sperm production as well as maintains sex drive following puberty.

The structures of the male and female reproductive system are adapted for the production and release of the gametes. In addition, the female reproductive system ensures a suitable location for fertilization and provides an environment for the growth of the embryo/foetus until birth.

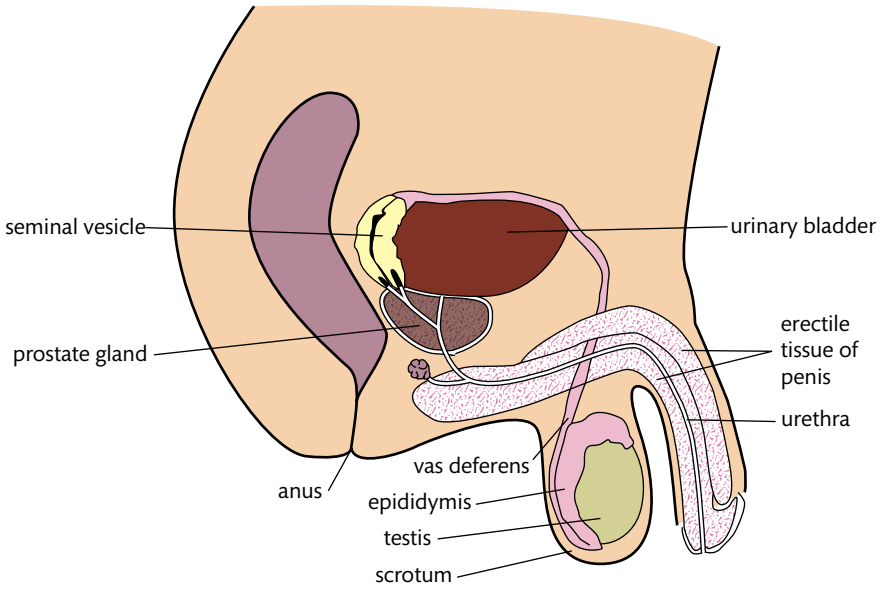
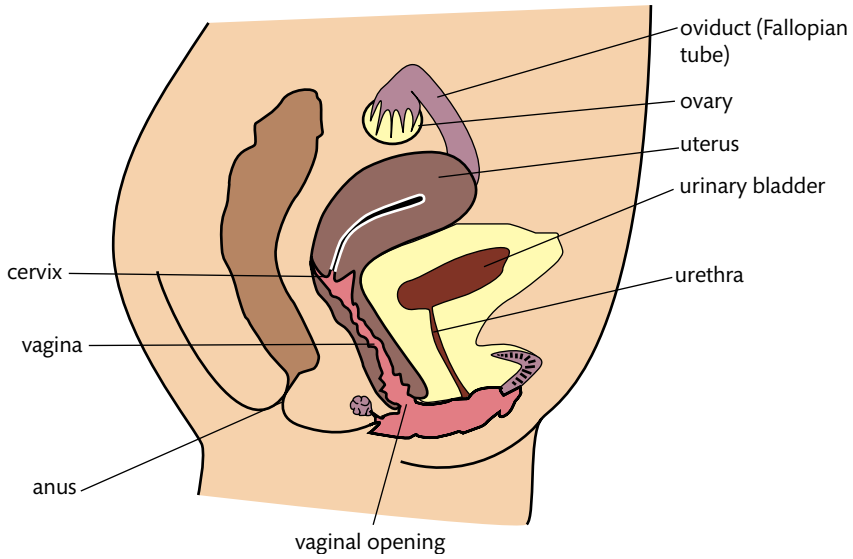


Figure 6.24 Male reproductive system (plus bladder).

Table 6.5 The male reproductive anatomy and function

Male structure	Function(s)
Testis	The male gonads: the sperm are produced here in small tubes called seminiferous tubules
Epididymis	The area where sperm are received, become mature, and are capable of swimming motion via movement of their flagella
Scrotum	Sacs that hold the testes outside the body cavity so that sperm production and maturation can occur at a temperature cooler than body temperature
Vas deferens	A muscular tube that carries mature sperm from the epididymis to the urethra during an ejaculation
Seminal vesicles	Small glands that produce and add seminal fluid to the semen
Prostate gland	A gland that produces much of the seminal fluid, including carbohydrates for the sperm
Penis	An organ that becomes erect as a result of blood engorgement in order to facilitate ejaculation
Urethra	After all the glands have added fluids, this is the tube via which the semen leaves the penis



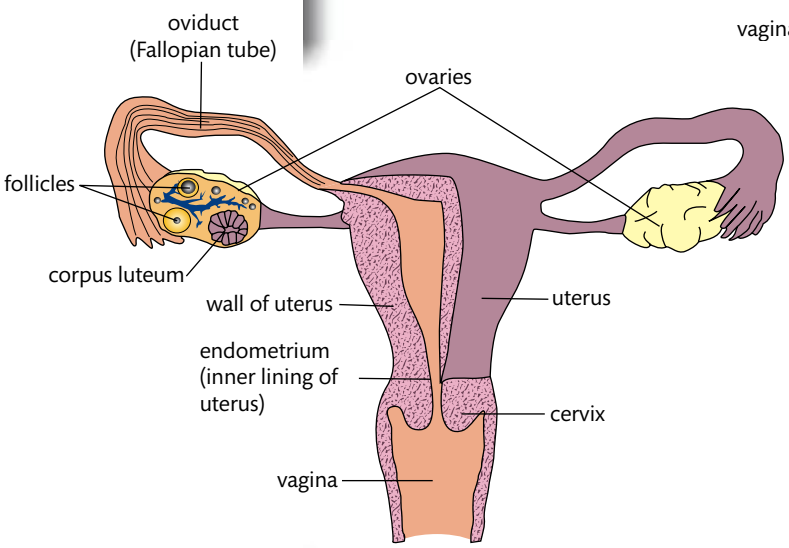


Figure 6.25 Female reproductive system from sagittal (top) and frontal (bottom) views.

Table 6.6 The female reproductive anatomy and function

Female structure	Function(s)
Ovaries	Organs that produce and secrete oestrogen. They also produce and release the ovum (in the form of secondary oocytes). The area where ovulation occurs grows into the corpus luteum, which temporarily produces the hormone progesterone
Fallopian tubes (oviducts)	Ducts that carry the ovum (or early embryo) to the uterus
Uterus	A muscular structure where the early embryo implants and develops if a pregnancy occurs
Endometrium	The highly vascular inner lining of the uterus
Cervix	The lower portion of the uterus, which has an opening to the vagina that allows the sperm to enter for fertilization and provides a pathway for childbirth
Vagina	A muscular tube that leads from the external genitals to the cervix; semen is ejaculated here during sexual intercourse

How does a person become male or female?

You will learn (or have already learnt) that the genetics of becoming male or female depends on whether you inherit an X or a Y chromosome from your father. Because your mother has two X chromosomes, an ovum can only contain an X chromosome. One half of all sperm cells contains an X and one half contains a Y chromosome. If a sperm cell containing an X chromosome fertilizes an ovum, a female is produced. Conversely, if a sperm cell containing a Y chromosome fertilizes an ovum, a male is produced.

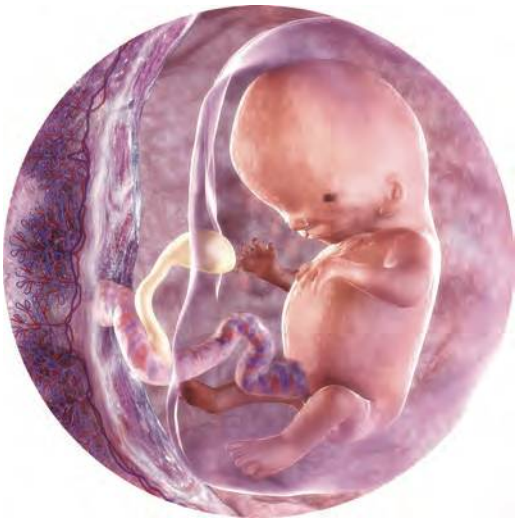
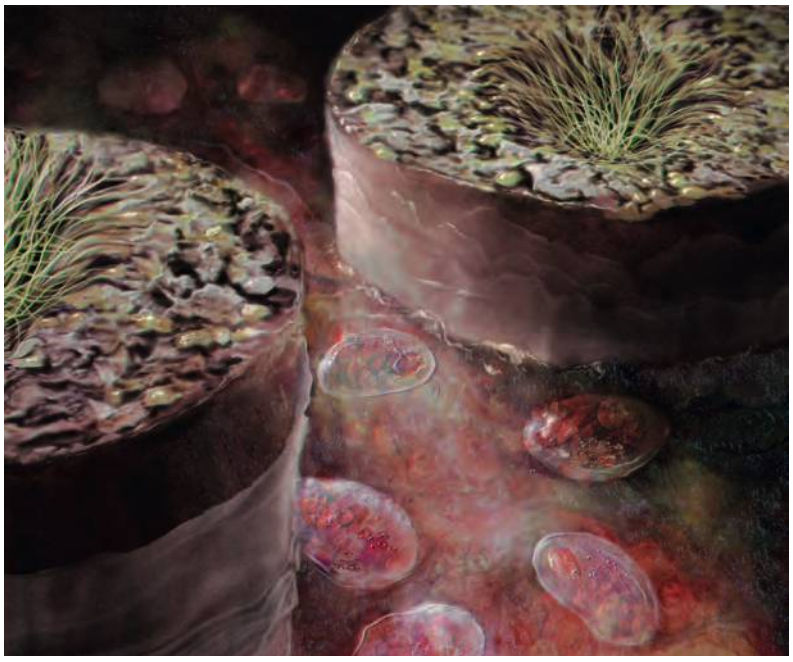


Illustration of a human 8-week-old embryo. The development of internal and external structures characteristic of the sex of the embryo begin about this time. A portion of the placenta is shown on the left, with the umbilical blood vessels within the umbilical cord stretching from the placenta to the embryo.

So, what happens as a result of the XX or XY combinations? The answer lies in the hormones that are produced by each embryo. Embryos of both sexes are virtually identical until about the eighth week following fertilization. Alleles that interact on both of the X chromosomes of female embryos then result in relatively high oestrogen and progesterone production, resulting in the prenatal development of female reproductive structures. Genes located on the single Y chromosome are responsible for early testes development and relatively high testosterone production, resulting in male reproductive structures during subsequent foetal development. It is interesting to note that the male and female reproductive structures have common origins in the pre-8-week-old embryo. In other words, the same embryonic tissue that becomes the ovaries gives rise to the testes, the same embryonic tissue that gives rise to the clitoris gives rise to portions of the penis, etc. Another way of expressing this is to say that some female and male reproductive structures are homologous.



Leydig cells in each testis produce testosterone. Leydig cells are found between the small tubules (seminiferous tubules) that produce spermatozoa (sperm cells). Two seminiferous tubules are shown in cross-section on the upper and left parts of the figure, with Leydig cells in between. Inside the seminiferous tubules you can see developing spermatozoa with flagella surrounded by cells in various stages of meiosis..

It was once assumed that embryos that produced testosterone changed from the 'default' sex of female to male. There is now evidence that each sex requires the influence of specific hormones in order to follow its pathway.

TOK

How much influence should a government have on family planning? A good example of government influence is the One Child Policy of the People's Republic of China. Some, but not all, couples are fined for having more than one child. The policy has had reasonable success as a population control measure, but is resulting in a disproportionately high percentage of males in certain areas of China.

Although males typically experience a lower sperm count as they age, fertility has been documented in men as old as 94 years.

NATURE OF SCIENCE

In Section 6.2 you learnt about the work of William Harvey and how he provided the first valid explanation of how blood circulates in the body. William Harvey was also responsible for much of the early knowledge of a branch of biology that we now call embryology. Embryology is the study of the early development of embryos from fertilized egg to birth. William Harvey's insights were considerable, but lacked information about the earliest embryonic development stages. This was because William Harvey carried out his studies before the microscope had been invented. William Harvey died 17 years before the invention of the microscope.

Role of sex hormones during puberty

When females and males reach puberty, the same hormones that first determined their physical sex are produced and secreted in higher amounts. The increased production of hormones at this time results in the secondary sex characteristics (the attributes that are characteristic of a sex that only appear at puberty).

The secondary sex characteristics of females that arise as a result of increased oestrogen and progesterone production at puberty are:

- enlargement of breasts
- growth of pubic and underarm hair
- widening of hips.

The secondary sex characteristics of males that arise as a result of increased testosterone production at puberty are:

- growth of facial, underarm, chest, and pubic hair
- enlargement of the larynx and associated deepening of the voice
- increased muscle mass
- enlargement of the penis.

The menstrual cycle

Starting at puberty, human females begin a hormonal cycle known as the menstrual cycle. Each cycle lasts, on average, 28 days. The purpose of the menstrual cycle is to time the release of an egg or ovum (ovulation) for possible fertilization and later implantation into the inner lining of the uterus. This implantation must occur when the uterine inner lining (the endometrium) is rich with blood vessels (i.e. highly vascular). The highly vascular endometrium is not maintained if there is no implantation. The breakdown of the blood vessels of the endometrium leads to the menstrual bleeding (menstruation) of a typical cycle. This menstruation is a sign that no pregnancy has occurred.

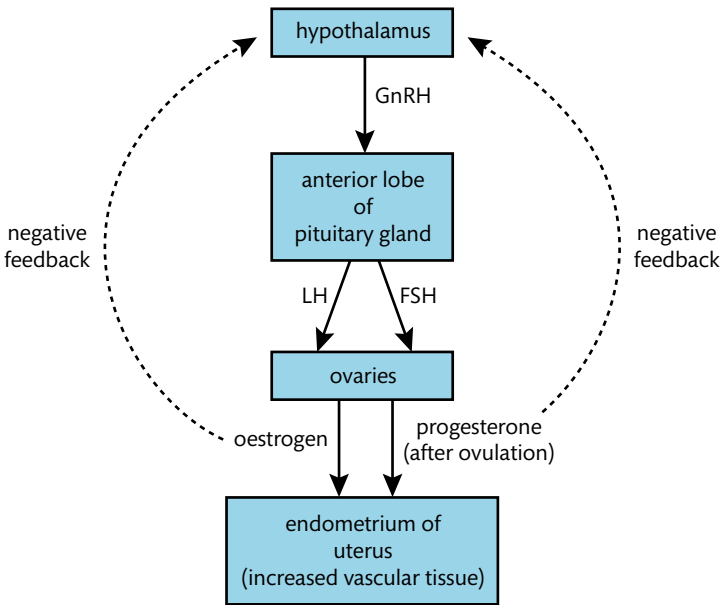


Figure 6.26 Hormonal summary of the menstrual cycle.

Hormones from the hypothalamus and pituitary gland

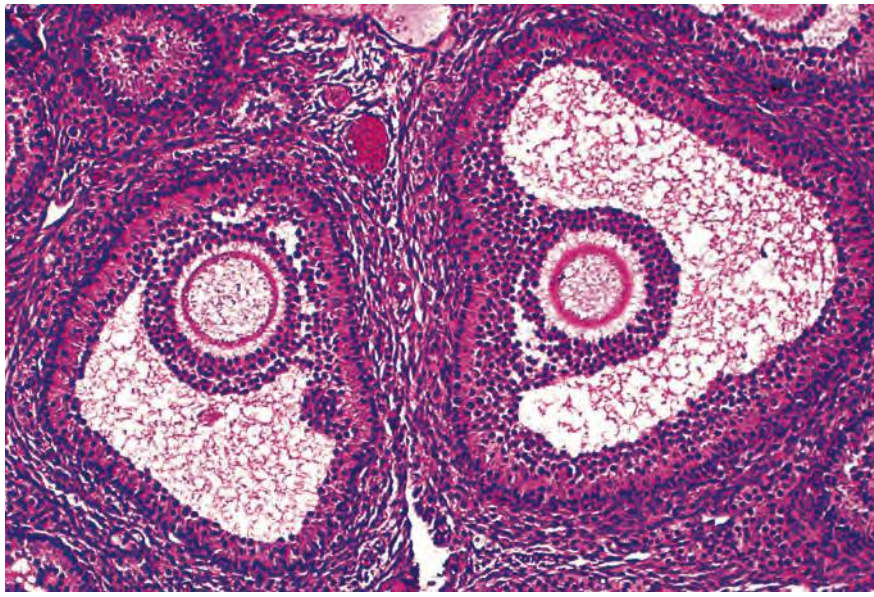
A part of a female's brainstem known as the hypothalamus is the regulatory centre of the menstrual cycle. The hypothalamus produces a hormone known as gonadotropin-releasing hormone (GnRH). The target tissue of GnRH is the nearby pituitary gland, and it results in the anterior pituitary producing and secreting two hormones into the bloodstream. These two hormones are follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The target tissues for these two hormones are the ovaries.

The effects of FSH and LH on the ovaries

The hormones FSH and LH have several effects on the ovaries. One of these effects is to increase the production and secretion of another reproductive hormone by the follicle cells of the ovary. This hormone is oestrogen. Like all hormones, oestrogen enters the bloodstream. Its target tissue is the endometrium of the uterus. One effect of oestrogen is an increase in the density of blood vessels of the endometrium, that is, as stated earlier, the endometrium becomes highly vascular. Another effect of oestrogen is to stimulate the pituitary gland to release more FSH and LH. This is the positive feedback loop of the menstrual cycle, specifically these two sets of hormones increasing because of the increase of the other(s).

Another effect of FSH and LH is the production of structures within the ovaries known as Graafian follicles. Within the ovaries are cells known as follicle cells, and the true reproductive cells that are at a stage of development called oocytes. Under the chemical stimulation of FSH and LH, the somewhat randomly arranged follicle cells and oocytes take on a cellular arrangement known as a Graafian follicle.

A spike in the level of FSH and LH leads to ovulation (the release of the oocyte from the Graafian follicle). The oocyte is accompanied by the inner ring of follicle cells of the Graafian follicle. This entire structure is known as a follicle, and typically enters the Fallopian tube soon after ovulation. The outer ring of follicle cells remains within the ovary. These follicle cells begin to produce and secrete another hormone, progesterone. The cells of this outer ring begin to divide and fill in the 'wound' area left by ovulation, and



A light micrograph showing a human ovary section. Two Graafian follicles are visible, with an oocyte at the centre of each (two inner circles).

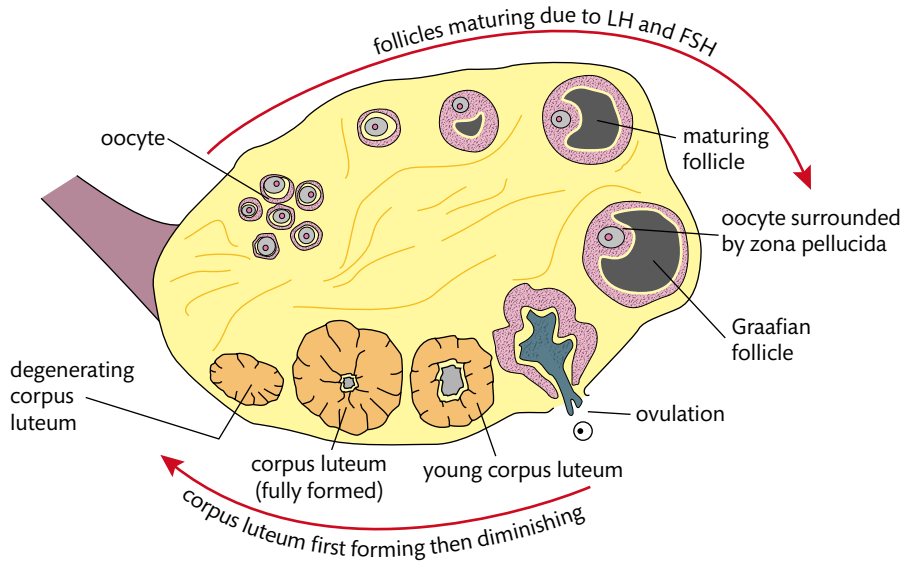


Figure 6.27 Ovary events during a single menstrual cycle. Twenty-eight days of ovarian events are being shown with a single ovary as if in time lapse.

Birth control pills contain both oestrogen and progesterone. Because these pills keep the levels of these two hormones high in a woman's bloodstream, the hypothalamus does not produce GnRH. Thus the pituitary does not produce FSH and LH, and no new Graafian follicles are produced within the ovaries. The end result is that ovulation does not occur.

this forms a glandular structure known as the corpus luteum. The corpus luteum will be hormonally active (producing progesterone) for only 10–12 days after ovulation. Progesterone is a hormone that maintains the thickened, highly vascular endometrium. As long as progesterone continues to be produced, the endometrium will not break down and an embryo will still be able to implant. In addition, the high levels of both oestrogen and progesterone at the same time provide a negative feedback signal to the hypothalamus. The hypothalamus does not produce GnRH when the oestrogen and progesterone levels are high, so FSH and LH remain at levels that are not conducive to the production of another Graafian follicle during this time.

Assuming there is no pregnancy, the corpus luteum begins to break down after 10–12 days, and this leads to a decline in both progesterone and oestrogen levels. As both of these hormone levels fall, the highly vascular endometrium can no longer be maintained. The capillaries and small blood vessels begin to rupture and menstruation begins. The drop in progesterone and oestrogen also signals the hypothalamus to begin secreting GnRH, and thus another menstrual cycle begins. Because the menstrual cycle is a cycle, there is no true beginning or ending point. The first day of menstruation is designated as the first day of the menstrual cycle simply because this is an event that can be easily discerned (see Figure 6.28).

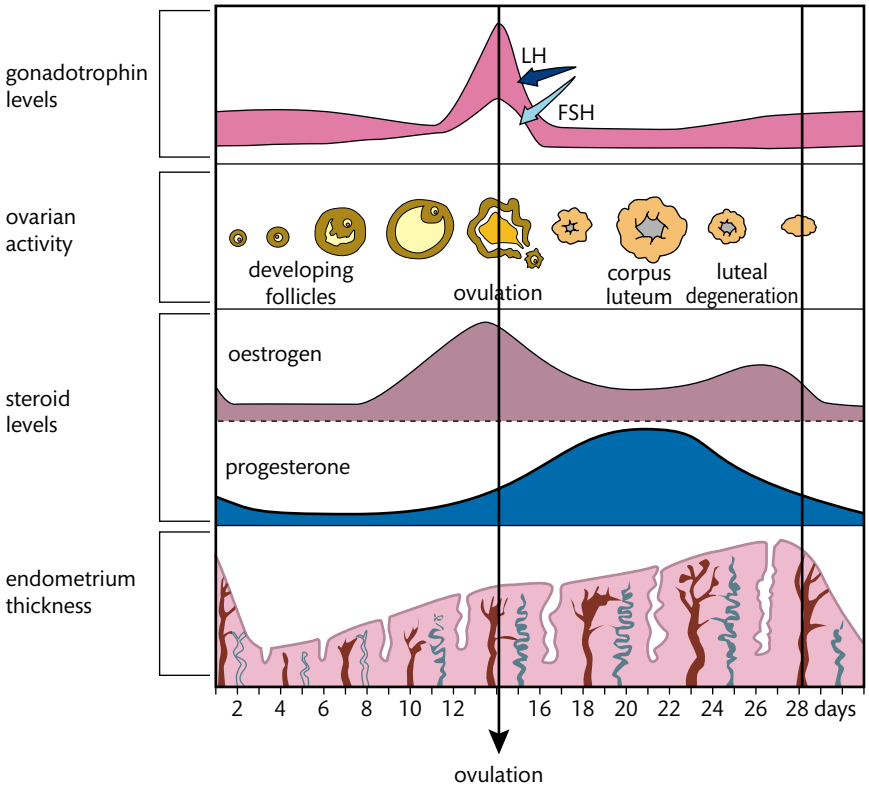


Figure 6.28 Events occurring during a 28-day menstrual cycle. Note that these events are all aligned on the same time scale. Ovulation and possible fertilization occur near the middle of the cycle.

When making sense of Figure 6.28, use the line indicating the time of ovulation as an important marker. Ask yourself: 'What events led up to and resulted in this ovulation?' and 'What will now happen after ovulation?'

In vitro fertilization (IVF)

Natural fertilization typically occurs in one of a female's Fallopian tubes 24–48 hours after ovulation. The resulting zygote begins to divide by mitosis, and takes several more days to travel down the Fallopian tube to the endometrium of the uterus. When the embryo reaches the endometrium, it has already divided mitotically many times and is a ball of about 100 cells. The embryo, called a blastocyst at this stage, will then implant in the highly vascular tissue of the endometrium.

Some couples are unable to bear children. There is a wide variety of possible reasons for infertility, including:

- males with low sperm counts
- males with impotence (failure to achieve or maintain an erection)
- females who cannot ovulate normally
- females with blocked Fallopian tubes.

Reproductive technologies have been developed to help overcome these situations. One of the most common of these new technologies is *in vitro* fertilization (IVF).

Hormone therapy

As part of the IVF procedure, a woman must have eggs 'harvested' from her ovaries. In order to ensure the proper timing for this, and to maximize the number of available ova, the woman undergoes about a month of hormone therapy. During the first 2 weeks she injects a drug (or uses a nasal spray of the drug) that suspends her own natural hormones associated with her menstrual cycle. Then for the next 12 days or so she takes hormone injections that include FSH. This ensures that she will produce many Graafian follicles in each ovary and provide many potential ova (oocytes) for harvesting. The production of many more eggs than is typical of a normal menstrual cycle is called superovulation.

When the time is right, several eggs (oocytes) are then harvested surgically. To obtain the sperm cells that are needed for fertilization, the man ejaculates into a container. Harvested eggs are mixed with the sperm cells in separate culture dishes. Microscopic observation reveals which ova are fertilized, and whether the early development appears normal and healthy. Between one and three healthy embryos are later introduced into the woman's uterus for implantation. Any healthy embryos from the culturing phase that are not implanted can be frozen and used later if another implantation procedure is needed.

TOK

Screening the embryos used in IVF for certain genetic conditions is becoming a common practice. Screening for desirable traits is possible, and may soon become a routine part of the IVF procedures offered by medical clinics. How much will the course of human evolution be effected by such screening practices?

NATURE OF SCIENCE

Always consider the source! If you do a web search for IVF, many of the sites you will encounter will be from private clinics that offer IVF as a paid service. This doesn't mean the information on those sites is incorrect, but it does mean you need to consider the possible bias behind the information.

Exercises

17 If possible, without looking back though this chapter, give a very brief description of the function of each of these hormones: insulin, glucagon, thyroxin, leptin, and melatonin.

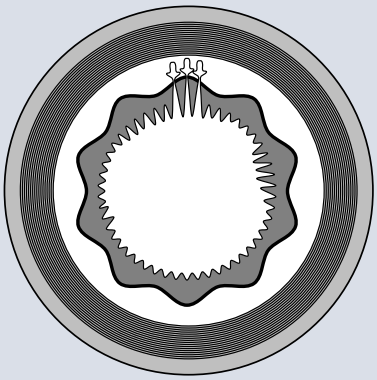
18 What is an example of a positive feedback loop in the menstrual cycle?

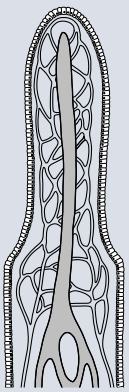
19 What is an example of a negative feedback loop in the menstrual cycle?

To learn more about this chapter, go to the hotlinks site, search for the title or ISBN and click on Chapter 6.

Practice questions

1 The first figure shows a cross-section through the small intestine, and the second figure shows an enlarged longitudinal section through a single villus.





Using these diagrams, outline **three** ways in which the structure of the small intestine is related to its function of absorbing food.

(*Total 3 marks*)

2 Draw a diagram of the human digestive system.

(*Total 4 marks*)

3 Explain the relationship between the structure and function of arteries, veins, and capillaries.

(*Total 9 marks*)

4 Explain why antibiotics are effective against bacteria but not viruses.

(*Total 3 marks*)

5 A blood clot contains a network of protein. What is the protein?

A Fibrin

B Fibrinogen

C Haemoglobin

D Thrombin

(*Total 1 mark*)

6 What happens during inhalation?

A Both the external intercostal muscles and the diaphragm contract.

B The internal intercostal muscles contract and the diaphragm relaxes.

C The external intercostal muscles relax and the diaphragm contracts.

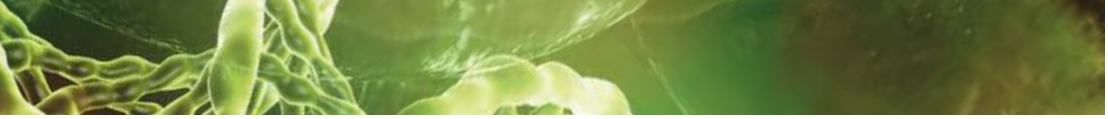
D Both the internal intercostal muscles and the diaphragm relax.

(*Total 1 mark*)

7 Describe the principles of synaptic transmission in the nervous system.

(*Total 6 marks*)

07 Nucleic acids



This diagram of a section of DNA represents an amazing molecule that is capable of coding for all the major characteristics of the unbelievably large number of organisms that have existed and that do exist on our planet today.

Essential ideas

7.1 The structure of DNA is ideally suited to its function.

7.2 Information stored as a code in DNA is copied onto mRNA.

7.3 Information transferred from DNA to mRNA is translated into an amino acid sequence.

The cells of all organisms are made up of a staggering number of molecules. These molecules include water, minerals, proteins, carbohydrates, lipids, nucleic acids, and many, many more. Of these molecules, proteins are of extreme importance because of their role in controlling the metabolism of cells, as mentioned in Section 2.5. If proteins are so important, then the importance of deoxyribonucleic acid (DNA) must also be recognized, as this very large macromolecule carries the code for making the proteins. Since the model of DNA was proposed in the early 1950s, DNA research has provided us with great advances, including the ability to detect tendencies for certain diseases in many organisms, the development of more productive plants and animals to feed a greater number of the world's population, and even the ability to attack certain types of cancer by allowing chemotherapies to be tailored to a specific organism's genetic makeup. This chapter will examine DNA replication as well as protein synthesis via transcription and translation.

7.1 DNA structure and replication

NATURE OF SCIENCE

Making careful observations: Rosalind Franklin's X-ray diffraction provided crucial evidence that DNA is a double helix.

Understandings:

- Nucleosomes help to supercoil the DNA.
- DNA structure suggested a mechanism for DNA replication.
- DNA polymerases can only add nucleotides to the 3′ end of a primer.
- DNA replication is continuous on the leading strand, and discontinuous on the lagging strand.
- DNA replication is carried out by a complex system of enzymes.
- Some regions of DNA do not code for proteins but have other important functions.

Applications and skills:

- Application: Rosalind Franklin and Maurice Wilkin's investigation of DNA by X-ray diffraction.
- Application: Use of nucleotides containing dideoxyribonucleic acid to stop DNA replication in preparation of samples for base sequencing.
- Application: Tandem repeats are used in DNA profiling.
- Skill: Analysis of results of the Hershey and Chase experiment to provide evidence that DNA is the genetic material.
- Skill: Utilization of molecular visualization software to analyse the association between protein and DNA within a nucleosome.

Guidance

- *Details of DNA replication differ between prokaryotes and eukaryotes. Only the prokaryotic system is expected.*
- *The protein enzymes involved in DNA replication should include helicase, DNA gyrase, single strand binding proteins, DNA primase, and DNA polymerases I and III.*
- *The regions of DNA that do not code for proteins should be limited to regulators of gene expression, introns, telomeres, and genes for tRNAs.*

Is DNA the genetic material?

In 1952, two researchers, Alfred Hershey and Martha Chase, carried out experiments that helped confirm DNA as the genetic material of life. They made use of radioisotopes in their experiment. Radioisotopes are radioactive forms of elements that decay over time at a predictable rate. The particles given off in this decay allow detection of the specific radioisotope used. They grew bacteriophage viruses in two different types of cultures. One culture included radioactive phosphorus-32, ^{32}P. The viruses produced in this culture had DNA in their core with the detectable phosphorus. Another culture included a radioactive form of sulfur known as sulfur-35, ^{35}S. This detectable radioisotope was present in the protein outer coat of the viruses produced in this culture. As DNA does not include sulfur, there was no ^{35}S inside the outer coat. The two types of bacteriophage with the different radioisotopes were then allowed to infect the bacterium known as *Escherichia coli*. As Figure 7.1 shows, the *E. coli* infected with the ^{32}P bacteriophage had radioactivity inside their cell wall. However, the *E. coli* infected with the ^{35}S had no radioactivity within their cell wall. Because DNA contains phosphorus and not sulfur, this allowed Hershey and Chase to conclude that DNA, not protein, was the genetic material of the bacteriophage. Prior to this, strong arguments were being presented that supported protein in this role. The experiment by Hershey and Chase used a bacteriophage known as T2 and the bacterium *E. coli*. A bacteriophage is a virus composed of a protein outer coat and an inner core of DNA or, sometimes, RNA. When this virus infects a cell, it takes over the metabolism of the cell, resulting in multiple viruses of its kind being formed. The Hershey–Chase experiment is illustrated in Figure 7.1. Once the results of the Hershey–Chase experiment were made known, research involving genetics centred on nucleic acids and, especially, DNA.

CHALLENGE YOURSELF

Using the results shown in Figure 7.1, answer the following questions.

1. Why were ^{32}P and ^{35}S used in the experiment?
2. What part of the bacteriophage is actually inserted into *E. coli*?
3. Did any protein from the bacteriophage enter the bacterium?
4. What can be concluded from this experiment?

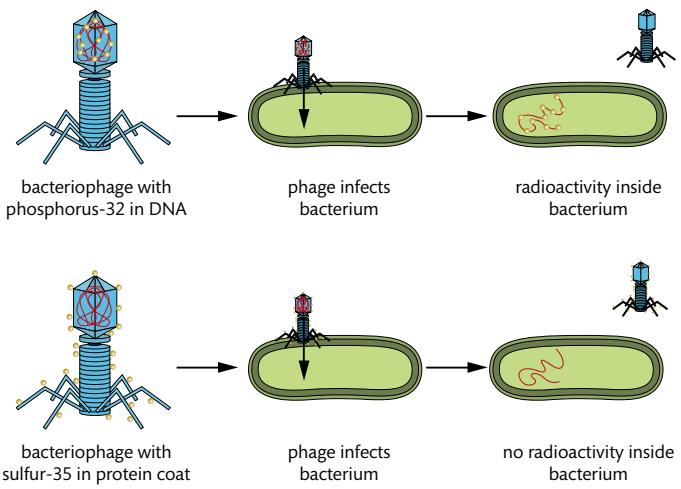


Figure 7.1 The Hershey–Chase experiment used radioisotopes as markers to label the DNA and protein of T2 bacteriophages. The basic procedure and findings are shown here.

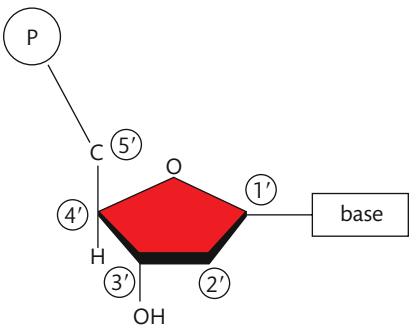


Figure 7.2 A DNA nucleotide is composed of a molecule of deoxyribose with a phosphate group attached to the 5′ (five-prime) carbon and a nitrogenous base attached to the 1′ (one-prime) carbon.

DNA structure

To understand the detailed structure of DNA, you must be familiar with the numbering of the carbon atoms in the pentose sugar of DNA, which is deoxyribose.

From Chapter 2, you already know that DNA is a double-stranded molecule formed in the shape of a double helix. This would be a good time for you to review what you learnt in Chapter 3 by drawing a generalized one-chain portion of DNA. Show the position of the covalent bonds between adjacent nucleotides. If you have difficulty doing this, you should review Section 2.6.

NATURE OF SCIENCE

Francis Crick and James Watson used information from many sources to produce their model of the structure of DNA. Pictures of DNA by Rosalind Franklin and Maurice Wilkins using X-ray crystallography, and conclusions from their findings, were used by Crick to conclude that the molecule was composed of two strands arranged as a double helix. These X-ray diffraction images also allowed an understanding of the width of the DNA molecule and the spacing of the nitrogenous bases within it. Science often makes major leaps forward when careful and critical observations are made.

How is a single chain of DNA made up?

Each strand of DNA is composed of a backbone of alternating phosphate and deoxyribose molecules. These two molecules are held together by a covalent bond called a phosphodiester bond or linkage. A phosphodiester bond in DNA forms between a hydroxyl group of the 3′ carbon of deoxyribose and the phosphate group attached to the 5′ carbon of deoxyribose.

The bonding between single nucleotides to produce a long chain is controlled by specific enzymes. These enzymes will only allow the chain to grow in a 5′ to 3′ direction.

So, each nucleotide is attached to the previous one by this type of bond. This produces a chain of DNA. The reaction between the phosphate group on the 5′ carbon and the hydroxyl group on the 3′ carbon is a condensation reaction, with a molecule of water released. When two nucleotides unite in this way, the two-unit polymer still has a 5′ carbon free at one end and a 3′ carbon free at the other end. Each time a nucleotide is added, it is attached to the 3′ carbon end. Even when thousands of nucleotides are involved, there is still a free 5′ carbon end with a phosphate group attached and a free 3′ carbon end with a hydroxyl group attached. This creates the alternating sugar–phosphate backbone of each chain.

As nucleotides are linked together with covalent phosphodiester bonds, a definite sequence of nitrogenous bases develops. This sequence carries the genetic code that is essential for the life of the organism.

How are the two strands of DNA held together?

The two sugar–phosphate backbones are attached to each another by their nitrogenous bases. The two backbones or chains run in opposite directions and are described as antiparallel. One strand has the 5′ carbon on the top and the 3′ carbon on the bottom; the other strand is the opposite way round (see Figure 7.3).

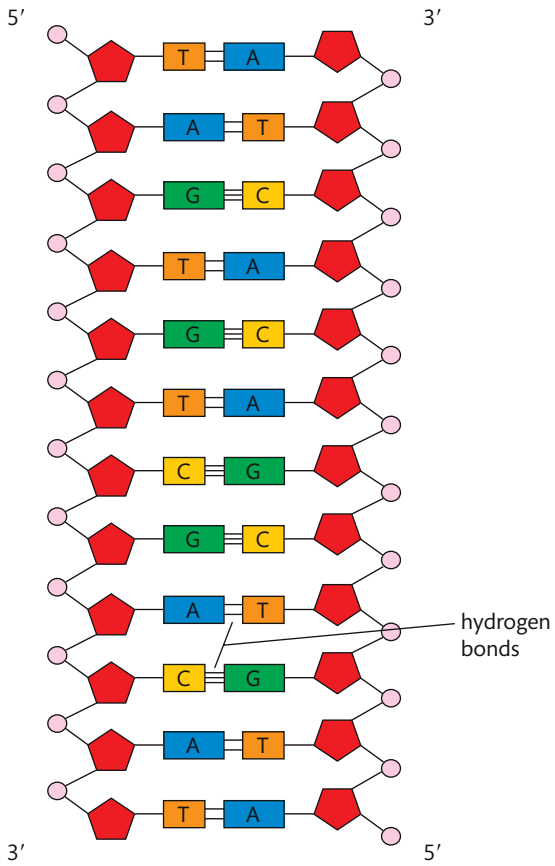


Figure 7.3 The antiparallel strands in DNA run in opposite directions.

The nitrogenous bases form links by means of hydrogen bonds. There are four nitrogenous bases: adenine (A), thymine (T), cytosine (C), and guanine (G). Adenine and guanine are double-ring structures known as purines. Cytosine and thymine are single-ring structures known as pyrimidines. A single-ring nitrogenous base always pairs with a double-ring nitrogenous base. This is complementary base pairing, and occurs because of the specific distance that exists between the two sugar–phosphate chains. Adenine always pairs with thymine (from the opposite chain), and cytosine always pairs with guanine (from the opposite chain). Hydrogen bonds link the two nitrogenous bases together: two hydrogen bonds link adenine and thymine; three hydrogen bonds link cytosine and guanine.

DNA nucleotides are composed of a pentose sugar, a phosphate group, and a nitrogenous base. The nitrogenous bases can be one of the purines (adenine and guanine), or one of the pyrimidines (thymine and cytosine).

DNA packaging

The DNA molecules of eukaryotic cells are paired with a type of protein called histone (see Figure 7.4). Actually, there are several histones, and each helps in DNA packaging. Packaging is essential because the nucleus is microscopic and a single human molecule of DNA in a chromosome may be 4 cm long.

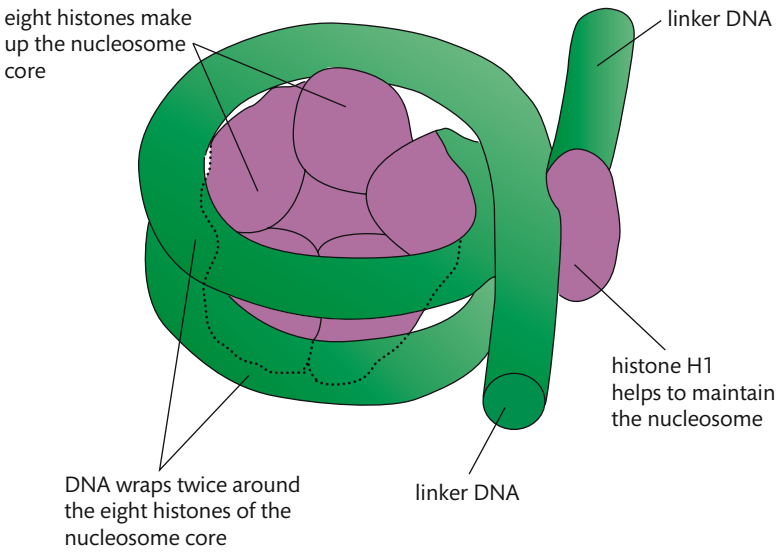
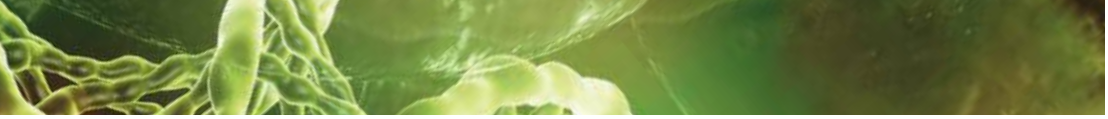


Figure 7.4 Histones and DNA together form nucleosomes.

When looking at unfolded DNA with an electron microscope, you can see what looks like beads on a string. Each of the beads is a nucleosome. A nucleosome consists of two molecules of each of four different histones. The DNA wraps twice around these eight protein molecules. The DNA is attracted to the histones because DNA is negatively charged and histones are positively charged. Between the nucleosomes is a single string of DNA. There is often a fifth type of histone attached to the linking string of DNA near each nucleosome. This fifth histone leads to further wrapping (packaging) of the DNA molecule, and eventually to highly condensed or supercoiled chromosomes.

When DNA is wrapped around the histones and then further wrapped in even more elaborate structures, it is inaccessible to transcription enzymes. Therefore, the wrapping or packaging of DNA brings about a regulation of the transcription process. This allows only certain areas of the DNA molecule to be involved in protein synthesis.



Types of DNA sequences

The genomes, complete DNA sequences, of many eukaryotes are now known because of rapid advancements in the field of genomics. Genomics involves the science of sequencing, interpreting, and comparing whole genomes. From the multinational Human Genome Project, we have learned that less than 2% of human DNA actually codes for proteins or other materials required for protein synthesis in the cell. Regions of DNA that do not code for proteins include areas that act as regulators of gene expression, introns, telomeres, and genes that code for transfer ribonucleic acids (tRNAs). This would be a good time to review Section 3.5, which discusses DNA profiling, polymerase chain reaction (PCR) and gel electrophoresis. A solid understanding of these processes will help you with the following information.

Highly repetitive sequences

A large amount of human DNA is composed of highly repetitive sequences. These repetitive sequences are usually composed of 5–300 base pairs per repetitive sequence. There may be as many as 100 000 replicates of a certain type per genome. If this repetitive DNA is clustered in discrete areas, it is referred to as satellite DNA.

However, this repetitive DNA is mostly dispersed throughout the genome. At the present time, as far as we can tell these dispersed regions of DNA do not have any coding functions. They are transposable elements, which means they can move from one genome location to another. These elements were first found by Barbara McClintock in 1950; in 1983, she received the Nobel Prize for her discovery. Even though these transposable elements, often referred to as jumping genes, are able to change their position within a chromosome, they never actually detach from the DNA molecule they are part of.

Protein-coding genes

Within the DNA molecule of a chromosome are the single-copy genes that have coding functions. They provide the base sequences essential to produce proteins at the cell ribosomes. Any base sequence is carried from the nucleus to the ribosomes by mRNA. Work to determine the complete base sequence of human chromosomes began in the mid 1970s. With the first information published from the Human Genome Project in 2001, it became apparent that less than 2% of the chromosomes were occupied by genes that code for protein.

A gene is not a fixed sequence of bases like the letters of a word. Genes are made up of numerous fragments of protein-encoding information interspersed with non-coding fragments. The coding fragments make up what are known as exons, while the non-coding fragments make up introns. Exons and introns are discussed in Section 7.2 regarding transcription and translation.

Structural DNA

Structural DNA is highly coiled DNA that does not have a coding function. It occurs around the centromere and near the ends of chromosomes at the telomeres. Some scientists refer to the sections of DNA that appear to not have a coding function as pseudogenes. Many feel these sections have lost their function due to a mutation involving a base sequence change.

The frequency of the various types of DNA sequence is shown in Table 7.1.

TOK

Labels are placed upon biological features just as they are often placed upon fellow human beings. For years the highly repetitive sequences found in certain samples of DNA were called 'junk DNA'. Discuss how this labelling may have affected DNA research. Are there valid arguments concerning the potential harm of labelling common to that which sometimes occurs for human beings and that which may occur for biological features?

With the Human Genome Project, biological research switched from an individualistic approach to that of large interdisciplinary teams encompassing engineering, informatics, and biology. The result was large-scale international cooperation. One outcome of this collaboration is the HapMap. This mapping project is enabling the discovery of genes related to many diseases, such as diabetes and cancer. Ultimately, this project may provide much more effective treatments for patients based upon their individual DNA composition.

Table 7.1 DNA sequences in the human genome

Sequence	Frequency (%)
Protein-encoding genes (exons)	1–2
Introns	24
Highly repetitive sequences	45
Structural DNA	20
Inactive genes (pseudogenes)	2
Other	7–8

The centromere of chromosomes is made up largely of highly repetitive sequences called satellite DNA. Structures called telomeres occur on the ends of chromosomes and they consist of a 6–8-base pair sequence that is repeated up to hundreds of thousands of times. The actual base pair sequence of telomeres and the number of repeats of this sequence varies with each species.

Short tandem repeats and DNA profiling

DNA profiling is the process of obtaining a specific DNA pattern from an organism, such as a human, from the body tissue of that organism. Most of our own DNA is identical to the DNA of other people. However, there are specific regions that show significant variation. These regions are referred to as polymorphisms. It is these polymorphisms that are analysed with DNA profiling. To profile polymorphisms, we often look at a group of 13 very specific loci (locations on a chromosome) referred to as short tandem repeats (STRs).

STRs are short, repeating sequences of DNA, normally composed of 2–5 base pairs.

Telomeres are presently a major area of research. Most of this research is centred on ageing and cancer.

CHALLENGE YOURSELF

5 A locus designated D7S280 is one of the 13 polymorphisms often used in DNA profiling. Below is a base sequence of this locus provided by GenBank, a public DNA database. The STR in this case is the tetramer 'gata'. The number to the left of the base sequences refers to the bases present.

```
  1 aatttttgta ttttttttag agacggggtt tcaccatgtt ggtcaggctg actatggagt
 61 tattttaagg ttaatatata taaagggtat gatagaacac ttgtcatagt ttagaacgaa
121 ctaacgatag atagatagat agatagatag atagatagat agatagatag atagacagat
181 tgatagtttt ttttatctc actaaatagt ctatagtaaa catttaatta ccaatatttg
241 gtgcaattct gtcaatgagg ataaatgtgg aatcgttata attcttaaga atatatattc
301 cctctgagtt tttgatacct cagattttaa ggcc
```

(a) What is abnormal in this case about the section marked, including STRs of 'gata'?
(b) How could individuals differ in this specific polymorphism?
(c) When would it be possible for two individuals to have exactly the same base sequence as above and even at the other 12 STR loci used in DNA profiling?

To learn more about GenBank, go to the hotlinks site, search for the title or ISBN, and click on Chapter 7: Section 7.1.

DNA profiling is useful in several situations, such as revealing family relationships, identifying individuals involved in a crime, and identifying disaster victims. The technique is quite reliable: if all 13 loci involving STRs are analysed, the likelihood of two individuals having the same profile is close to 1 in 1 billion, with the exception of identical twins.

DNA replication

When Watson and Crick proposed their model for the structure of DNA, they realized that the A–T and C–G base pairing provided a way for DNA to be copied. They thought that a single strand of DNA could serve as a template for a copy. This would facilitate the accuracy that is necessary to pass on the DNA information from one generation to the next. They referred to this idea as a semi-conservative model of DNA replication. The experiments carried out in the late 1950s by Matthew Meselson and Franklin Stahl confirmed this DNA semi-conservative model of replication. Refer to Section 2.7 to review the procedure and findings. Below is a figure representing the Meselson–Stahl experiment.

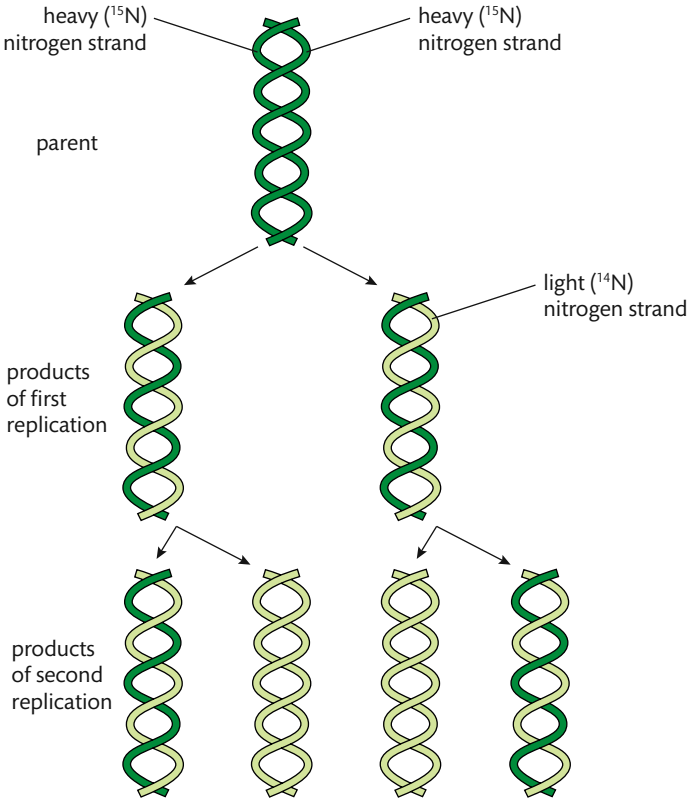


Figure 7.5 Bacteria grown in a medium with heavy nitrogen (^{15}N) have DNA that contains only heavy nitrogen. The bacteria are then placed in a medium with only light nitrogen (^{14}N). After one replication of DNA, the resulting DNA contains both light and heavy nitrogen. After another replication, the DNA is either all light or hybrid.

Semi-conservative replication

The process of DNA replication involving bacteria was developed as a result of Meselson and Stahl's experiment. The replication of DNA begins at special sites called origins of replication. Bacterial DNA is circular, has no histones, and has a single origin. Eukaryotic DNA is linear, has histones, and has thousands of origins. The presence of multiple replication origins greatly accelerates the copying of large eukaryotic chromosomes.

Because the bacterial DNA molecule is much smaller than eukaryotic DNA molecules, only one origin is necessary for replication of the bacterial chromosome.

Here is a brief summary of the replication process (see Figure 7.6).

1. Replication begins at the origin, which appears as a bubble because of the separation of the two strands. The separation or 'unzipping' occurs because of the action of the enzyme helicase on the hydrogen bonds between nucleotides.
2. At each end of a bubble there is a replication fork. This is where the double-stranded DNA opens to provide the two parental DNA strands that are the templates necessary to produce the daughter DNA molecules by semi-conservative replication.
3. The bubbles enlarge in both directions, showing that the replication process is bidirectional. The bubbles eventually fuse with one another to produce two identical daughter DNA molecules.

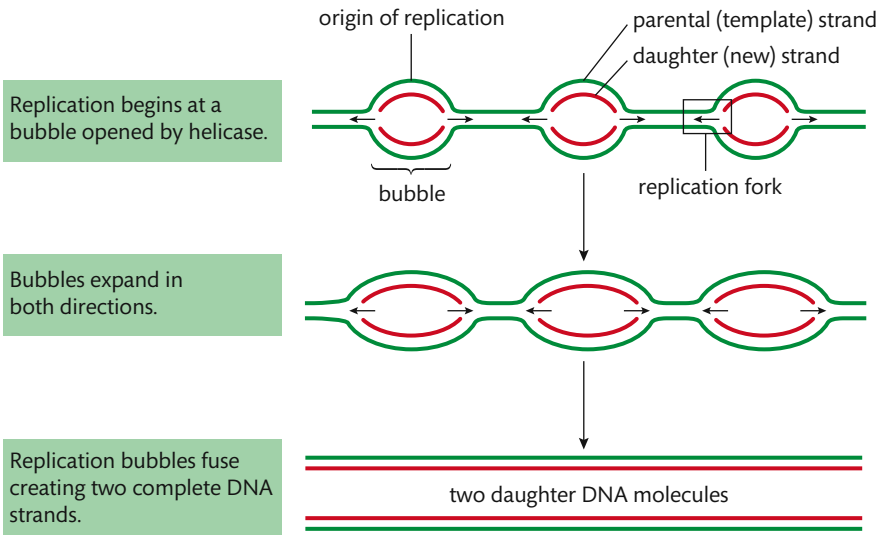


Figure 7.6 Semi-conservative replication means that each parental DNA strand acts as a template to form a complementary strand so eventually two identical daughter DNA molecules are formed. Eukaryotic DNA molecules are quite long and replication begins at a large number of sites along the molecule. This allows replication to occur at a much faster rate.

Elongation of a new DNA strand

The production of a new strand of DNA using the templates exposed at the replication forks occurs in an orderly manner.

1. A primer is produced under the direction of primase at the replication fork. This primer is a short sequence of RNA, usually only 5–10 nucleotides long. Primase allows the joining of RNA nucleotides that match the exposed DNA bases at the point of replication.
2. The enzyme DNA polymerase III then allows the addition of nucleotides in a 5′ to 3′ direction to produce the growing DNA strand.
3. DNA polymerase I also participates in the process. It removes the primer from the 5′ end and replaces it with DNA nucleotides.

Each nucleotide that is added to the elongating DNA chain is actually a deoxynucleoside triphosphate (dNTP) molecule. This molecule contains deoxyribose, a nitrogenous base (A, T, C, or G), and three phosphate groups. As these molecules are added, two phosphates are lost. This provides the energy necessary for the chemical bonding of the nucleotides.

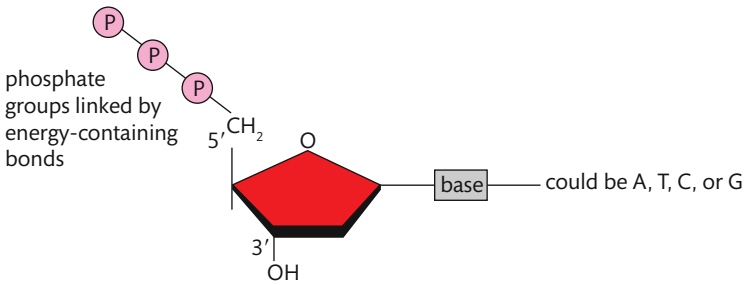


Figure 7.7 This is a generalized deoxynucleoside triphosphate molecule.

The antiparallel strands

A DNA molecule is composed of two antiparallel strands. One strand is 5′ to 3′ and the other is 3′ to 5′. DNA strands can only be assembled in the 5′ to 3′ direction because of the action of polymerase III. Therefore there is a difference in the process of

assembling the two new strands of DNA from the templates. For the 3′ to 5′ template strand, the new DNA strand is formed as described above. The process is continuous and relatively fast, and the strand produced is called the **leading strand**. The other new strand forms more slowly and is called the **lagging strand**.

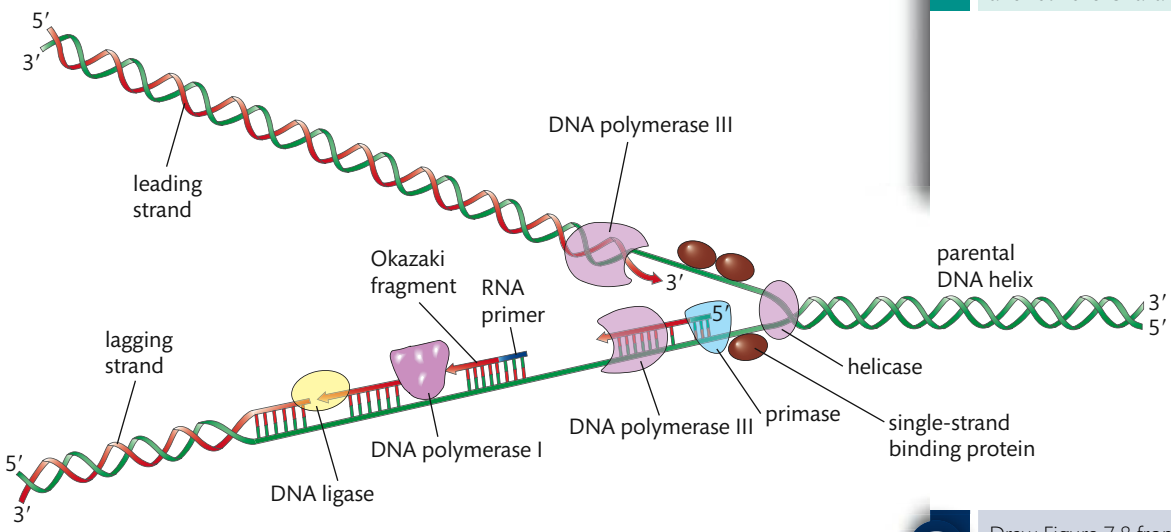
Formation of the lagging strand involves fragments and an additional enzyme called **DNA ligase** (see Figure 7.8).

1. The leading strand is assembled continuously towards the progressing replication fork in the 5′ to 3′ direction.
2. The lagging strand is assembled by fragments being produced moving away from the progressing replication fork in the 5′ to 3′ direction.
3. The fragments of the lagging strand are called Okazaki fragments, after the Japanese scientists who discovered them.
4. Primer, primase, and DNA polymerase III are required to begin the formation of each Okazaki fragment of the lagging strand, and to begin the formation of the continuously produced leading strand.
5. The primer and primase are only needed once for the leading strand because it is produced continuously.
6. Once the Okazaki fragments are assembled, an enzyme called DNA ligase attaches the sugar–phosphate backbones of the lagging strand fragments to form a single DNA strand.

The leading and lagging strands are assembled concurrently. However, there is a slight delay in the synthesis of the lagging strand.

Prior to the mid 1960s, research from scientists at various facilities around the world seemed to indicate that DNA replication was continuous on both strands. However, that changed in 1966 when a team of Japanese scientists studying DNA replication in *E. coli* found that one strand of DNA was involved in a discontinuous process in which fragments were produced and linked later. Several scientists at different locations around the world produced research indicating this discontinuous strand formation. However, the final evidence involving enzymes and fragments was published by a team that included Rejii Okazaki and Tsuneko Okazaki.

Figure 7.8 At a replication fork, helicase separates the strands of the double helix and binding proteins stabilize the single strands. There are two mechanisms for replication: continuous synthesis and discontinuous synthesis. Continuous synthesis occurs on the leading strand: primase adds an RNA primer and DNA polymerase III adds nucleotides to the 3′ end of the leading strand. DNA polymerase I then replaces the primer with nucleotides. Discontinuous synthesis occurs on the lagging strand: primase adds an RNA primer in front of the 5′ end of the lagging strand and DNA polymerase III adds nucleotides. DNA polymerase I replaces the primer, and finally DNA ligase attaches the Okazaki fragment to the lagging strand.



Draw Figure 7.8 from memory and annotate on the drawing what is happening at specific locations.

Replication proteins

The basic processes of DNA replication were worked out with research using *E. coli*. Table 7.2 summarizes the roles of the replication proteins in *E. coli*.

Table 7.2 The roles of the replication proteins in *E. coli*

Protein	Role
Helicase	Unwinds the double helix at replication forks
Primase	Synthesizes RNA primer
DNA polymerase III	Synthesizes the new strand by adding nucleotides onto the primer (in a 5′ to 3′ direction)
DNA polymerase I	Removes the primer and replaces it with DNA
DNA ligase	Joins the ends of DNA segments and Okazaki fragments

In Table 7.2 notice that all the proteins end with the suffix –ase. This indicates that these proteins are acting as enzymes, speeding up a specific reaction without themselves changing.

Research involving eukaryotes has shown that replication in prokaryotic and eukaryotic cells is almost identical. In eukaryotic cells, the enzyme DNA gyrase stabilizes the DNA double helix when helicase unzips the molecule at multiple sites.

Speed and accuracy of replication

Even though this process seems quite complicated, it occurs at a phenomenal rate: up to 4000 nucleotides are replicated per second. This speed is essential in cells such as bacteria, which may divide every 20 minutes. Eukaryotic cells contain huge numbers of nucleotides compared with prokaryotic cells. To accomplish rapid DNA replication in these cells, multiple replication origins are needed.

Replication of DNA is remarkably accurate. Few errors (mutations) occur, which is stunning given the huge numbers of the nucleotides that are replicated. Cells have a number of repair enzymes that detect and correct errors when they do occur. These repair enzymes are also used when chemicals or high-energy waves cause damage to existing cells.

Replication, DNA sequencing, and the Human Genome Project

Earlier in this chapter, DNA profiling was described as a reliable means of identifying personal characteristics. The Human Genome Project required more than identification of DNA, it required an actual representation of the nucleotide sequence in humans. This involved the sequencing of DNA. In the 1970s, Frederick Sanger developed the first sequencing procedure. It used fragments of DNA copied through a process called polymerase chain reaction (PCR). PCR allows large numbers of copies to be made of a DNA fragment. These copies are denatured (separated into single strands) by heating them in a solution at 92°–94°C. Sequencing then begins.

1. Single-stranded fragments are placed in four different test tubes. All test tubes contain the primers, the DNA polymerases necessary to begin DNA replication, and the nucleotides of DNA.
2. Each tube contains a special nucleotide called dideoxynucleotide, derived from a dideoxynucleic acid (ddNTP). This nucleotide, after being added by DNA polymerase, prevents any further nucleotide addition to the chain. There are four

different dideoxynucleotides, just as there are four different nucleotides. Therefore, one tube contains A* (adenine dideoxynucleotide, corresponding to the adenine nucleotide), T* (thymine dideoxynucleotide, and so on), C*, and G*.

3 Synthesis of each new DNA strand then begins at the 3′ end of the primer, and continues until a dideoxynucleotide is added. These dideoxynucleotides are only available in limited amounts but allow chains of various lengths to be assembled.

4 DNA from each tube is placed in a separate lane of an electrophoresis gel and electrophoresis is carried out. The bands produced in each lane may then be used to determine the exact sequence of that particular fragment of DNA.

CHALLENGE YOURSELF

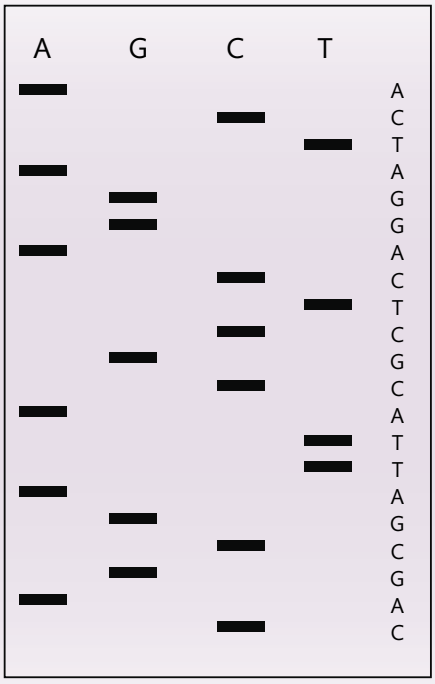


Figure 7.9 An example of Sanger's manual method of DNA sequencing. http://agctsequencing.wordpress.com/2012/08/01/sanger-sequencing-historical-development-of-automated-dna-sequencing/

6 Figure 7.9 shows results using the Sanger method of DNA sequencing. What would be the nucleotide sequence of this DNA fragment if the bases represent the origin of migration in the gel?

Newer methods of DNA sequencing are faster and cheaper. However, dideoxyribonucleic acid and ddNTPs are still used. The chain-stopping unique dideoxynucleotides are now labelled with fluorescent markers for easy recognition and quicker sequencing. The easier and quicker sequencing methods used now are based on Sanger's original technique. These methods allowed faster results with the Human Genome Project. They have also allowed fast and accurate mapping of the genomes of other types of organisms.

Exercises

1 Draw the two strands of a DNA molecule representing their antiparallel relationship.
2 Explain how nucleosomes contribute to transcription control.
3 Besides the nucleus, what other DNA source exists in eukaryotic cells that can be used for profiling?
4 What is the energy source for the production of the complementary strand of DNA?
5 What effect would only one origin of replication on a eukaryotic chromosome have on the cell cycle?
6 Compare the number of primers needed on the leading and the lagging strands of DNA in replication.

To learn more about the Human Genome Project, DNA structure and GenBank, go to the hotlinks site, search for the title or ISBN, and click on Chapter 7: Section 7.1.

NATURE OF SCIENCE

Looking for patterns, trends, and discrepancies: there is mounting evidence that the environment can trigger heritable changes in epigenetic factors.

7.2 Transcription and gene expression

Understandings:

- Transcription occurs in a 5′ to 3′ direction.
- Nucleosomes help to regulate transcription in eukaryotes.
- Eukaryotic cells modify mRNA after transcription.
- Splicing of mRNA increases the number of different proteins an organism can produce.
- Gene expression is regulated by proteins that bind to specific base sequences in DNA.
- The environment of a cell and of an organism has an impact on gene expression.

Applications and skills:

- Application: The promoter as an example of non-coding DNA with a function.
- Skill: Analysis of changes in the DNA methylation patterns.

Guidance

- *RNA polymerase adds the 5′ end of the free RNA nucleotide to the 3′ end of the growing mRNA molecule.*

Is there a central dogma of molecular biology?

DNA is sequestered (locked away) in the nucleus. Ribosomes are in the cytoplasm. But the two need to get together for the vital process of protein synthesis to occur. So how does the DNA code get to the ribosomes? The code is carried from the nucleus by the second type of nucleic acid: ribonucleic acid (RNA).

The set of ideas first proposed by Francis Crick in 1956 called the central dogma states that information passes from genes on the DNA to the RNA copy. The RNA copy then directs the production of proteins at the ribosome by controlling the sequence of amino acids. This mechanism is one-way and fundamental to all forms of life. The central dogma can be summarized as:

$$\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}$$

The process that occurs at the first arrow in the central dogma is transcription. The process that occurs at the second arrow is translation.

Even though scientists have found exceptions to the central dogma, the basic idea, that genetic information flows in this general direction, has not been invalidated.

CHALLENGE YOURSELF

7 In Section 7.1, the packaging of DNA into nucleosomes was explained. DNA may exist in two forms, chromatin and chromosomes. Both forms are composed of DNA and the histone proteins. However, chromosomes are highly organized and compacted because of the large increase in nucleosomes. Chromatin does not have nearly as many nucleosomes. The chromatin phase of DNA is present in the cell when it is not dividing. This is when the cell is carrying out the functions necessary for life.

(a) Thinking about the structure of a nucleosome, will the DNA that is wrapped around the histones be involved in the transcription process?

(b) Thinking about the structure of a chromosome, which has many nucleosomes, what DNA would be available for the transcription process?

(c) If a gene that is on the DNA wrapped around the histones needs to be transcribed, what will have to happen to it?

Transcription: DNA → RNA

Transcription has some similarities with replication. For both processes, the double helix must be opened to expose the base sequence of the nucleotides. In replication, helicase unzips the DNA and both strands become templates for the formation of two daughter strands of DNA. However, in transcription, helicase is not involved. Instead, an enzyme called RNA polymerase separates the two DNA strands. The RNA polymerase also allows polymerization of RNA nucleotides as base pairing occurs along the DNA template. To provide these functions, the RNA polymerase must first combine with a region of the DNA strand called a promoter. You will recall that, in DNA replication, DNA polymerase allows assembly only in a 5′ to 3′ direction. The same is true with RNA polymerase. The 5′ ends of free RNA nucleotides are added to the 3′ end of the RNA molecule being synthesized.

To learn more about DNA replication, transcription, and translation, go to the hotlinks site, search for the title or ISBN, and click on Chapter 7: Section 7.2.

RNA polymerase binds to the promoter region of DNA. This causes the DNA to unwind. The polymerase then initiates the synthesis of an RNA molecule in a 5′ to 3′ direction.

But which strand of DNA is copied?

One strand is complementary to the other, so there is a difference in the code of the strands. The codons are specific for certain amino acids or are start or stop messages. The start or stop codons are also known as punctuation codons. The codons are specific for certain amino acids or punctuation signals. Therefore, complementary strands mean different codons, different amino acids, and, finally, different proteins.

The DNA strand that carries the genetic code is called the sense strand (or the coding strand). The other strand is called the antisense strand (or the template strand). The sense strand has the same sequence as the newly transcribed RNA, except with thymine in place of uracil. The antisense strand is the strand that is copied during transcription. Look at Figure 7.10. Note that RNA polymerase is the enzyme that breaks the hydrogen bonds to produce the two separate strands of DNA. Recent research indicates there is an enzyme with helicase activity that works with the RNA polymerase to open the DNA double helix. However, its exact structure has not been confirmed.

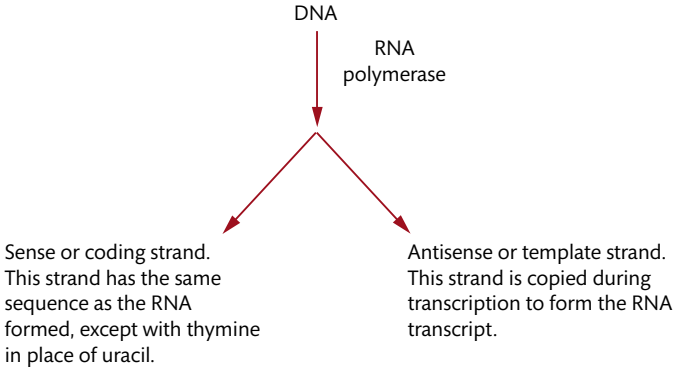


Figure 7.10 DNA strands in the transcription process.

The promoter region for a particular gene determines which DNA strand is the antisense strand. For any particular gene, the promoter is always on the same DNA strand. However, for other genes, the promoter may very well be on the other strand. The promoter region is a short sequence of bases that is not transcribed.

Once RNA polymerase has attached to the promoter region for a particular gene, the process of transcription begins. The DNA opens and a transcription bubble forms. This bubble contains the antisense DNA strand, the RNA polymerase, and the growing RNA transcript (see Figure 7.11).

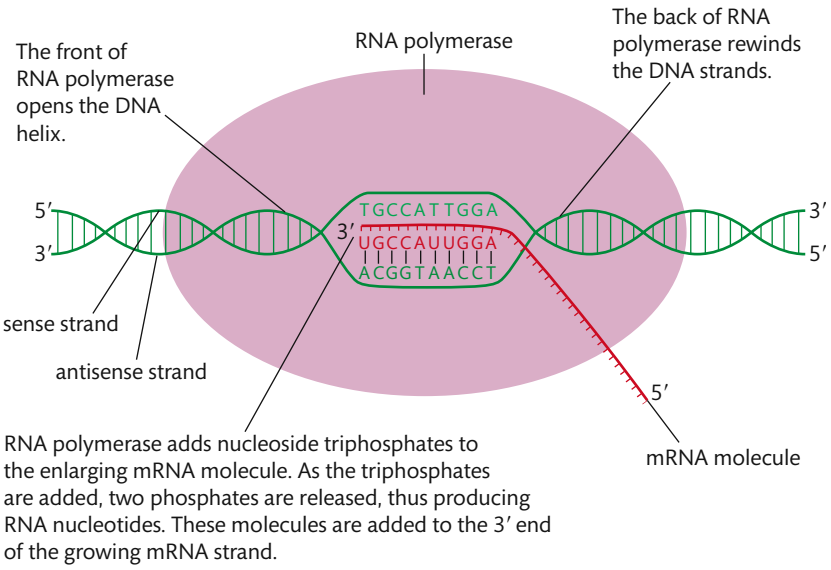


Figure 7.11 DNA is opened into two strands by RNA polymerase. The sense strand has the same base sequence as the new messenger (m) RNA. The antisense strand is the template for transcription, so the new mRNA has a base sequence that is complementary to it.

The promoter in the transcription process is a prime example of a non-coding section of DNA with a function.

The terminator

The sections of DNA involved in transcription are:

promoter → transcription unit → terminator

The transcription bubble moves from the DNA promoter region towards the terminator.

The terminator is a sequence of nucleotides that, when transcribed, causes the RNA polymerase to detach from the DNA. When this happens, transcription stops and the RNA transcript is detached from the DNA. The transcript carries the code of the DNA and is referred to as messenger RNA (mRNA).

In eukaryotes, transcription continues beyond the terminator for a significant number of nucleotides. Eventually, the transcript is released from the DNA strand.

Nucleoside triphosphates (NTPs), containing three phosphates and the 5-carbon sugar ribose, are paired with the appropriate exposed bases of the antisense strand. Polymerization of the mRNA strand occurs, with the catalytic help of RNA polymerase and the energy provided by the release of two phosphates from NTP. This portion of the transcription process is often referred to as elongation.

Post-transcription modification of mRNA

Eukaryotic cell DNA is different from prokaryote DNA in that within the protein-coding regions there are stretches of non-coding DNA. The stretches of non-coding DNA are called introns. As a complete region of a DNA molecule is transcribed to form mRNA, the first RNA formed is called pre-mRNA or the primary RNA transcript. It contains both exons and introns. To make a functional mRNA strand in eukaryotes, the introns are removed. The process by which the introns are removed is referred to as splicing. Those sequences of mRNA remaining after splicing are called exons. See Figure 7.12.

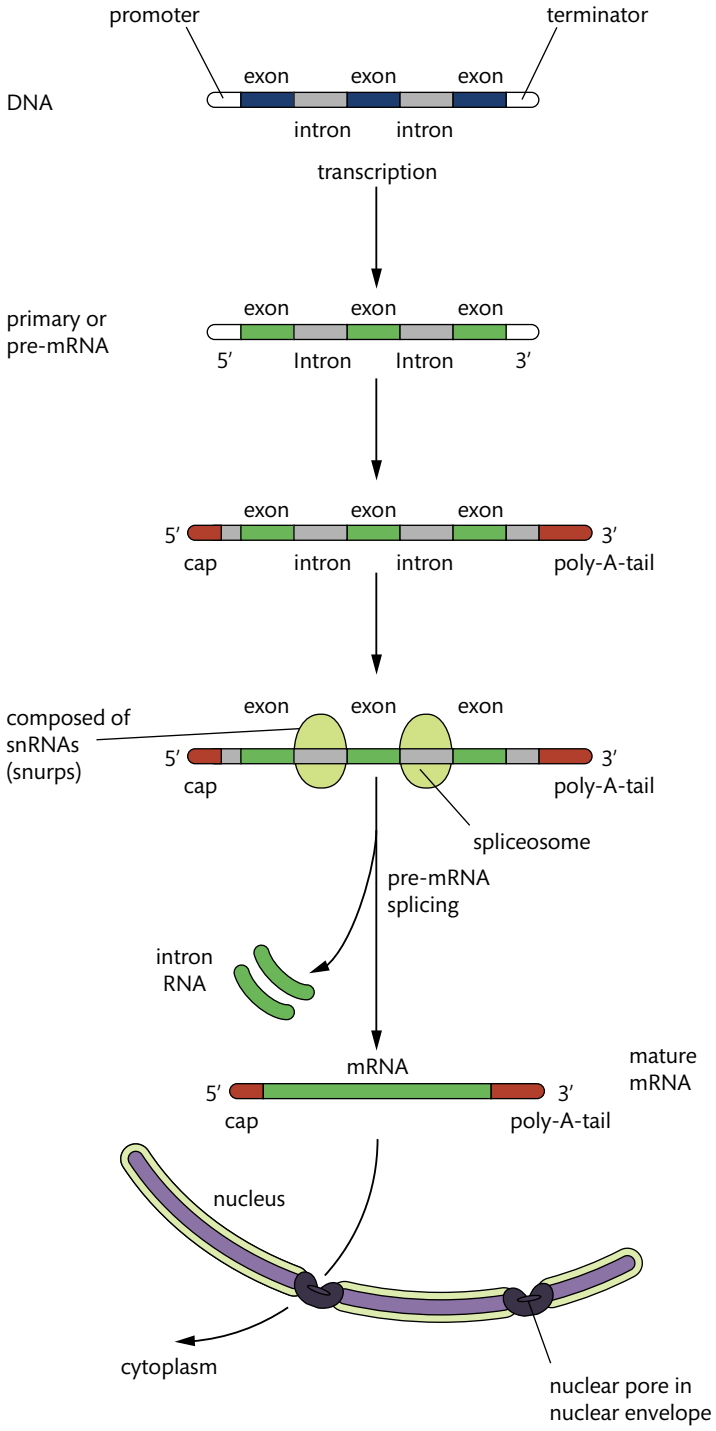


Figure 7.12 Splicing of mRNA in eukaryotes. DNA contains both introns and exons. Both of these are transcribed in the production of pre-mRNA (the primary mRNA transcript). During splicing the introns are removed, exons may be 'rearranged', and a cap and a poly-A tail (see page 338) are added to the ends of what is now called mature mRNA. It is this mature mRNA that moves out of the nucleus to the cytoplasm where a protein will be produced.

In Figure 7.12, the spliceosomes are composed of small nuclear RNAs (snRNAs, pronounced snurps). When the spliceosomes remove the introns, the exons may be rearranged, resulting in different possible proteins. In some higher eukaryotes different sections of a gene act as introns at different times. Again, this increases the number of possible proteins produced by one gene.

Biologists at one time firmly believed in the one gene–one protein concept. We now know a gene may produce several, if not many, different proteins. Splicing provides the mechanism for this to occur. This mechanism explains the surprisingly low number of genes found in the Human Genome Project.

Prokaryotic mRNA does not require processing because no introns are present.

On one end (the 5′ end) of the final mRNA transcript (mature mRNA), there is a cap made of a modified guanine nucleotide with three phosphates. The other end (the 3′ end) is fitted with a poly-A tail, which is composed of 50–250 adenine nucleotides. The cap and poly-A tail seem to protect the mature mRNA from degradation in the cytoplasm and to enhance the translation process that occurs at the ribosome.

Methylation and gene expression

Methylation may regulate the expression of either the maternal or paternal form (allele) of a gene. The methyl group appears to cause a section of DNA to wrap more tightly around histones, thus preventing transcription of that particular allele.

When observing DNA, it is apparent that inactive DNA is usually highly methylated compared with DNA that is actively being transcribed. A methyl group is an organic functional group with the formula CH_3. An example of this occurs in mammalian females. In female body cells, usually one of the X chromosomes becomes inactive. This inactivated X chromosome can be seen to be heavily methylated. Also, genes that are more heavily methylated are not usually transcribed or expressed. Once a gene has been methylated, it usually will stay that way even through many cell divisions. This allows methylation patterns to be maintained.

Unique methylation patterns have been associated with a large number of human cancers. These patterns include both hypermethylation (many methyl groups present compared with normal tissue) and hypomethylation (very few methyl groups present compared with normal tissue).

Methylation patterns are now being used in the diagnosis and treatment of some cancers. Prostate cancer is one example where certain detectable methylation patterns are being used in early diagnoses to classify tumours and promote successful treatments.

TOK

For many years, debates have addressed the importance of innate attributes and abilities versus those learned through experience. This is often referred to as the nature versus nurture argument. Discuss with your peers the values of each method of acquiring abilities in the survival of an organism. Why is science involved in this debate?

Proteins and gene expression

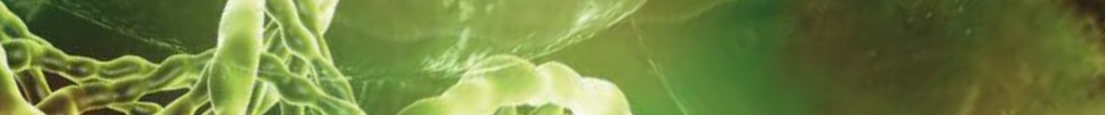
In many cases, gene expression is also regulated by proteins. Every cell appears to have many different types of transcription factors. These are proteins that regulate transcription by assisting the binding of RNA polymerase at the promoter region of a gene. Another type of protein that has an effect on gene expression is called a transcription activator. Transcription activators cause looping of DNA, which results in a shorter distance between the activator and the promoter region of the gene. This will bring about gene expression. There are also repressor proteins that bind to segments of DNA called silencers. This prevents transcription of the segment of that particular region.

Epigenetics involves the study of a set of reversible heritable changes that occurs without a change in the DNA nucleotide sequence. Therefore epigenetics involves the study of splicing, methylation, proteins, and also the environment and its effect on gene expression.

The environment and gene expression

Another area of gene expression involves the effect of the environment. Several recent studies have provided evidence that people with the same genotype will express different phenotypes when in different environments.

One recent study conducted by Youssef Idaghdour and Greg Gibson showed that many more respiratory genes are expressed in human populations living in urban areas than in agrarian areas. They concluded that pollutants in the urban areas stimulate the problems of diseases such as asthma and bronchitis because of the expression of usually non-expressed genes. Other environmental factors that affect gene expression are being researched.



Exercises

7 If the mRNA transcript is forming in the 5′ to 3′ direction, in what direction is the transcription bubble moving on the DNA antisense strand?

8 What type of mRNA requires processing? Explain why.

9 If a segment of DNA (gene) is examined and there are many more methyl groups present than in other genes, what will probably happen to the protein that that gene normally produces?

10 What organisms enable effective studies of epigenetics?

To learn more about epigenetics, go to the hotlinks site, search for the title or ISBN, and click on Chapter 7: Section 7.2.

7.3 Translation

Understandings:

- Initiation of translation involves assembly of the components that carry out the process.
- Synthesis of the polypeptide involves a repeated cycle of events.
- Disassembly of the components follows termination of translation.
- Free ribosomes synthesize proteins for use primarily within the cell.
- Bound ribosomes synthesize proteins primarily for secretion or for use in lysosomes.
- Translation can occur immediately after transcription in prokaryotes due to the absence of a nuclear membrane.
- The sequence and number of amino acids in the polypeptide is the primary structure.
- The secondary structure is the formation of alpha helices and beta pleated sheets stabilized by hydrogen bonding.
- The tertiary structure is the further folding of the polypeptide stabilized by interactions between R-groups.
- The quaternary structure exists in proteins with more than one polypeptide chain.

Application and skills:

- Application: tRNA-activating enzymes illustrate enzyme–substrate specificity and the role of phosphorylation.
- Skill: Identification of polysomes in electron micrographs of prokaryotes and eukaryotes.
- Skill: The use of molecular visualization software to analyse the structure of eukaryotic ribosomes and a tRNA molecule.

Guidance

- *Names of the tRNA binding sites are expected, as well as their roles.*
- *Examples of stop and start codons are not required.*
- *Polar and non-polar amino acids are relevant to the bonds formed between R-groups.*
- *Quaternary structure may involve the binding of a prosthetic group to form a conjugated protein.*

NATURE OF SCIENCE

Developments in scientific research follow improvements in computing: the use of computers has enabled scientists to make advances in bioinformatics applications such as locating genes within genomes and identifying conserved sequences.

Ribosomes

Once mRNA is produced from the DNA template, the process of actually producing the protein at the ribosomes can begin.

This process is referred to as translation because it changes the language of DNA to the language of protein. The centre of this process is the ribosome. Therefore, you need to understand the structure of this organelle.

Ribosomes can be seen with an electron microscope. Each ribosome consists of a large subunit and a small subunit. The subunits are composed of ribosomal RNA (rRNA) molecules and many distinct proteins. rRNA proteins are generally small and are associated with the core of the RNA subunits. Roughly two-thirds of their mass is rRNA. The molecules of the ribosomes are constructed in the nucleolus of eukaryotic

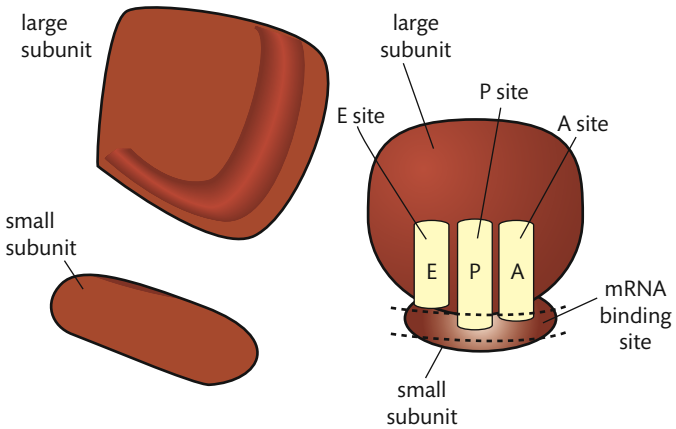
cells and exit the nucleus through the membrane pores. Prokaryotic ribosomes are smaller than eukaryotic ribosomes. There is also a difference in molecular makeup.

The decoding of a strand of mRNA to produce a polypeptide occurs in the space between the two subunits. In this area, there are binding sites for mRNA and three sites for the binding of tRNA, as shown in Table 7.3.

Table 7.3 Ribosomal binding sites for tRNA and their functions

Site	Function
A	Holds the tRNA carrying the next amino acid to be added to the polypeptide chain
P	Holds the tRNA carrying the growing polypeptide chain
E	Site from which tRNA that has lost its amino acid is discharged

Figure 7.13 This model shows the arrangement of subunits and binding sites in a ribosome.



The triplet bases of the mRNA codon pair with the complementary bases of the triplet anticodon of the tRNA.

Polypeptide chains are assembled in the cavity between the two subunits. This area is generally free of proteins, so binding of mRNA and tRNA is carried out by the rRNA. tRNA moves sequentially through the three binding sites: from the A site, to the P site, and finally to the E site. The growing polypeptide chain exits the ribosome through a tunnel in the large subunit core.

Translation: RNA → protein

The translation process involves several phases:

- initiation
- elongation
- translocation
- termination.

Before we discuss these phases, it is important to consider the codons. You will recall that codons carry the genetic code from DNA to the ribosomes via mRNA. There are 64 possible codons. Three codons have no complementary tRNA anticodon: these are the stop codons. There is a start codon (AUG) that signals the beginning of a polypeptide chain. This codon also encodes the amino acid methionine. The following table shows the meaning of the 64 different possible codons.

FIRST POSITION	SECOND POSITION: U	SECOND POSITION: C	SECOND POSITION: A	SECOND POSITION: G	THIRD POSITION
U	Phenyl alenine	Serine	Tyrosine	Cysteine	U
					C
	Lencine		Stop	Stop	A
			Stop	Tryptophan	G
C	Lencine	Proline	Histidine	Arginine	U
					C
			Glutamine		A
					G
A	Isoleucine	Threonine	Asparagine	Serine	U
					C
	*Methionine		Lysine	Arginine	A
					G
G	Valine	Alanine	Aspartic acid	Glycine	U
					C
			Glutamic acid		A
					G

Table 7.4 The genetic code. The first, second, and third positions represent the base location in the codon. Twenty amino acids are coded for. Note AUG is the start codon. Also, note the three stop or termination codons.

*And start.

From this table several points should be noted. The genetic code is degenerate, which means that, for each amino acid, there may be more than one codon. Also, the genetic code is universal, which means that, with only a few minor exceptions, all organisms share the same code. It is this universal aspect of the code that allows the exchange of genes from one species to another with the use of genetic engineering. Genetic engineering enabled us to place the human insulin-coding gene into bacteria so that the bacteria can produce this protein for human use.

Initiation of translation involves assembly of the components that carry out the process. Upon completion of the translation process, a disassembling of the involved components occurs.

The initiation phase

The start codon (AUG) is on the 5′ end of all mRNAs. Each codon, other than the three stop codons, attaches to a particular tRNA. The tRNA has a 5′ and a 3′ end, like all other nucleic acid strands. The 3′ end of tRNA is free and has the base sequence CCA. This is the site of amino acid attachment. Because there are complementary bases in the single-stranded tRNA, hydrogen bonds form at four areas. This causes the tRNA to fold and take on a three-dimensional structure. If the molecule is flattened, it has a two-dimensional appearance resembling a three-leaf clover. One of the loops of the clover leaf contains an exposed anticodon. This anticodon is unique to each type of tRNA. It is this anticodon that pairs with a specific codon of mRNA.

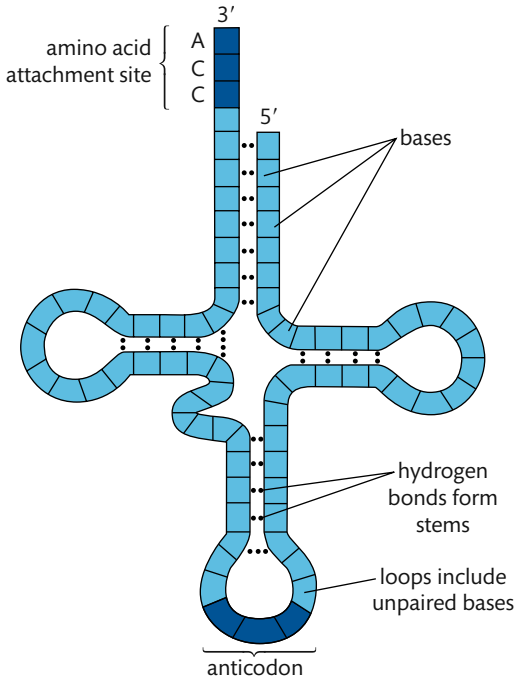


Figure 7.14 The two-dimensional clover-leaf structure of tRNA, with three loops. The anticodon triplet is unique to each tRNA.

The 20 enzymes that bind amino acids to tRNAs are grouped together and collectively called tRNA-activating enzymes.

Each of the 20 different amino acids will bind to the appropriate tRNA because of the action of a particular enzyme. Because there are 20 amino acids, there are 20 enzymes. The active site of each enzyme allows a fit only between a specific amino acid and the specific tRNA. The actual attachment of the amino acid and tRNA requires energy that is supplied by ATP. At this point, the structure is referred to as an activated amino acid, and the tRNA may now deliver the amino acid to a ribosome to produce the polypeptide chain.

So, the first step in initiation of translation is when an activated amino acid, methionine attached to a tRNA with the anticodon UAC, combines with an mRNA strand and a small ribosomal subunit.

The triplet bases of the mRNA codon form complementary base pairs with the triplet anticodon of the tRNA.

The small subunit moves down the mRNA until it contacts the start codon (AUG). This contact starts the translation process. Hydrogen bonds form between the initiator tRNA and the start codon. Next, the large ribosomal subunit combines with these parts to form the translation initiation complex. Joining the initiation complex are proteins called initiation factors that require energy from guanosine triphosphate (GTP) for attachment. GTP is an energy-rich compound very similar to ATP.

The elongation phase

Once initiation is complete, elongation occurs. This phase involves tRNAs bringing amino acids to the mRNA–ribosomal complex in the order specified by the codons of the mRNA. Proteins called elongation factors assist in binding the tRNAs to the exposed mRNA codons at the A site. The initiator tRNA moves to the P site. The ribosomes catalyse the formation of peptide bonds between adjacent amino acids that are brought to the polypeptide assembling area.

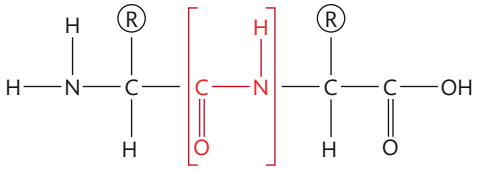


Figure 7.15 A peptide bond, shown in red in this figure, forms when water is given off. This process is called condensation.

The translocation phase

The translocation phase actually happens during the elongation phase. Translocation involves the movement of the tRNAs from one site of the mRNA to another. First, a tRNA binds with the A site. Its amino acid is then added to the growing polypeptide chain by a peptide bond. This causes the polypeptide chain to be attached to the tRNA at the A site. The tRNA then moves to the P site. It transfers its polypeptide chain to the new tRNA, which moves into the now exposed A site. The now empty tRNA is transferred to the E site, where it is released. This process occurs in the 5′ to 3′ direction. Therefore, the ribosomal complex is moving along the mRNA towards the 3′ end. Remember, the start codon was near the 5′ end of the mRNA.

The anticodon that moves into the A site is specific for the codon of the mRNA at that position. This allows the formation of a specific protein.

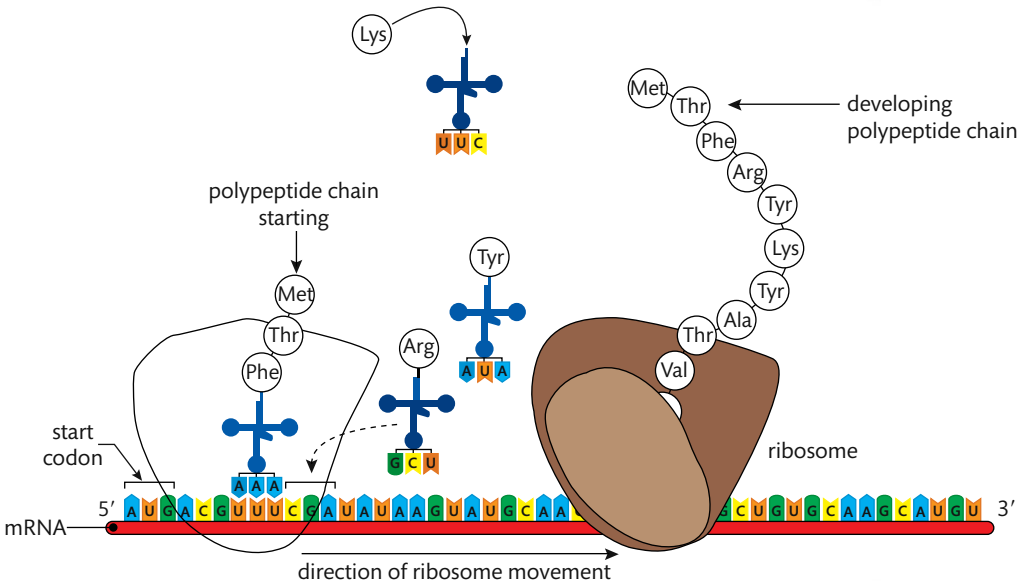


Figure 7.16 As the ribosome moves towards the 3′ end of the mRNA, the amino acid chain is assembled.

The termination phase

The termination phase begins when one of the three stop codons appears at the open A site. A protein called a release factor then fills the A site. The release factor does not carry an amino acid. It catalyses hydrolysis of the bond linking the tRNA in the P site with the polypeptide chain. This frees the polypeptide, releasing it from the ribosome. The ribosome then separates from the mRNA and splits into its two subunits.

The termination phase completes the process of translation. At this point, a disassembly process occurs in which the mRNA detaches from the ribosome, all tRNAs detach from the mRNA–ribosomal complex, and the protein is released from the ribosome. Proteins synthesized in this manner have several different destinations. If they are produced by free ribosomes, the proteins are primarily used within the cell. However, if the proteins are produced by ribosomes bound to the endoplasmic reticulum (ER), they are primarily secreted from the cell or used in lysosomes.

It is common to see multiple ribosomes going through the process of translation on a single mRNA strand. This string of ribosomes is called a polysome or polyribosome.

How can the environment affect characteristics supposedly determined specifically by transcription and translation? Should all individuals in all parts of the world have access to the development of positive environmental influences on their genome? What could be factors that stand in the way of this equal access?

TOK

Now that we have concluded our discussion of cellular protein synthesis, it would be helpful for you to summarize the major events in a table. Produce a table with two columns with the headings Transcription and Translation. Fill in as many major events as possible under each heading. Add as many rows as needed to complete it. Tables such as this are valuable in summarizing detailed information about a particular concept. Once your table is complete, you could compare your table with others in your class and discuss any variations.

To learn more about protein structures, go to the hotlinks site, search for the title or ISBN, and click on Chapter 7: Section 7.3.

Protein functions and structures

We have spent a lot of time discussing protein production in the cell, because proteins are very important. They serve many functions in cells and organisms. Table 7.5 shows you just a few examples of proteins and their functions.

Table 7.5 Examples of proteins and their functions

Protein example	Function
Haemoglobin	A protein containing iron that transports oxygen from the lungs to all parts of the body in vertebrates
Actin and myosin	Proteins that interact to bring about muscle movement (contraction) in animals
Insulin	A hormone secreted by the pancreas that helps maintain blood glucose level in many vertebrates
Immunoglobulins	A group of proteins that act as antibodies to fight bacteria and viruses
Amylase	A digestive enzyme that catalyses the hydrolysis of starch

There are proteins that have a structural role, proteins that store amino acids, and proteins that have receptor functions so that cells can respond to chemical signals. With all these functions, proteins have to be capable of assuming many forms and structures. The function of a protein is closely tied to its structure. There are four levels of organization to protein structure: primary, secondary, tertiary, and quaternary.

Primary organization

The primary level of protein structure refers to the unique sequence of amino acids. There are 20 different amino acids that are used to produce the proteins of organisms, and these can be arranged in any order. This order or sequence is determined by the nucleotide base sequence in the DNA of an organism. Because every organism has its own DNA, so every organism has its own unique proteins. The primary structure is simply a chain of amino acids attached by peptide bonds. Polypeptide chains can include hundreds of amino acids.

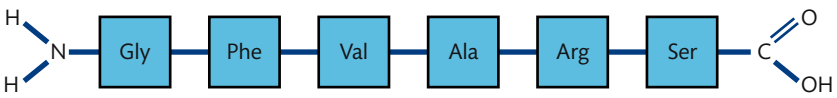


Figure 7.17 This figure represents a primary structure. Each blue box represents a particular amino acid. The lines connecting these amino acids represent a covalent bond called a peptide bond. The peptide bond is formed between an amino group of one amino acid and the carboxyl group of the other amino acid.

The primary structure determines the next three levels of protein organization. Changing one amino acid in a chain may completely alter the structure and function of a protein. This is what causes sickle cell disease. With this condition, just one amino acid has been changed in the normal protein (haemoglobin) of red blood cells. The result is that the red blood cells are unable to carry oxygen, which is their normal function.

Secondary organization

The next level in the organization of proteins is the secondary structure. This is created by the formation of hydrogen bonds between the oxygen from the carboxyl group of one amino acid and the hydrogen from the amino group of another. The secondary structure does not involve the side chains, the R-groups. The two most common configurations of the secondary structure are the alpha-helix (α-helix) and the beta-pleated sheet (β-pleated sheet). Both have regular repeating patterns.

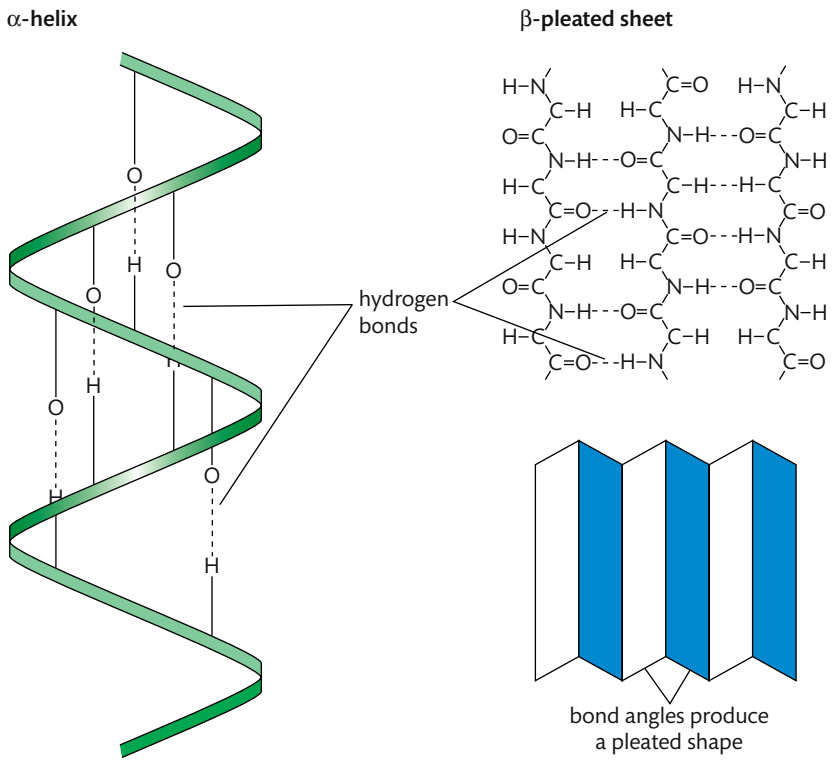


Figure 7.18 Protein secondary structure.

Transthyretin occurs in the silk protein of spider webs. It contains many β-pleated sheets that add to the strength of the web.

Tertiary organization

The third level in protein organization is the tertiary structure. The polypeptide chain bends and folds over itself because of interactions among the R-groups and the peptide backbone. This results in a definite three-dimensional conformation.

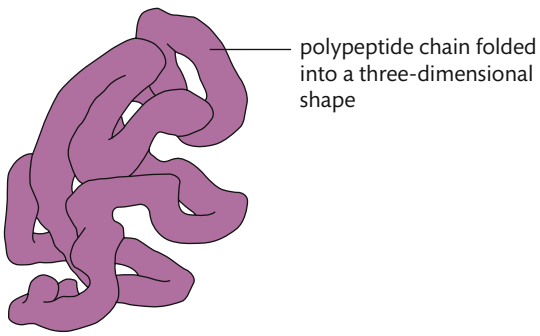


Figure 7.19 This is called a sausage model. It shows the three-dimensional conformation of lysozyme, an enzyme present in sweat, saliva, and tears. Lysozyme destroys many bacteria.

Interactions that cause tertiary organization include the following.

1 Covalent bonds between sulfur atoms to create disulfide bonds. These are often called bridges because they are strong bonds.

2 Hydrogen bonds between polar side chains.
3 Van der Waals interactions between hydrophobic side chains of amino acids. These interactions are strong because many hydrophobic side chains are forced inwards when the hydrophilic side chains interact with water towards the outside of the molecule.
4 Ionic bonds between positively and negatively charged side chains.

The tertiary structure is particularly important in determining the specificity of proteins that are enzymes.

Quaternary organization

The last level is the quaternary structure**.** This structure is unique in that it involves multiple polypeptide chains that combine to form a single structure.

Not all proteins consist of multiple chains, so not all proteins have a quaternary structure. All the bonds mentioned in the first three levels of protein structure are involved in this level. Some proteins with a quaternary level structure include prosthetic or non-polypeptide groups. These proteins are called conjugated proteins. Haemoglobin is a conjugated protein. It contains four polypeptide chains, each of which contains a non-polypeptide group called a haem. Haem contains an iron atom that binds to oxygen.

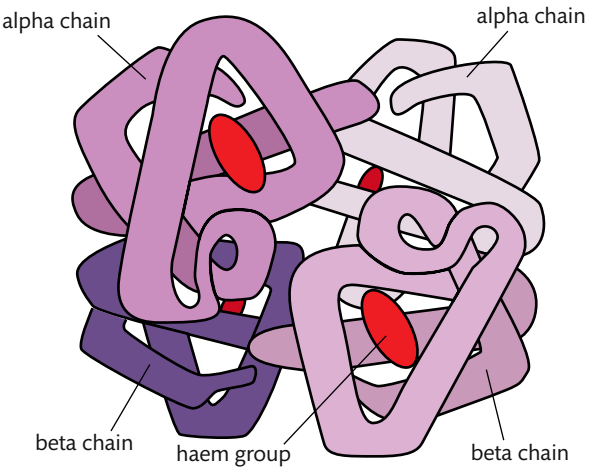
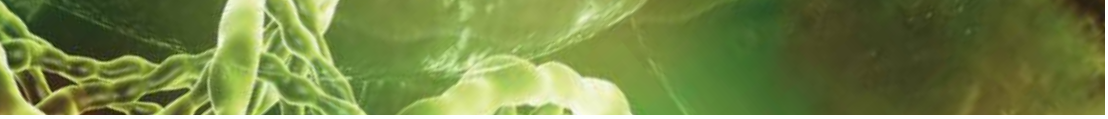


Figure 7.20 A sausage model of haemoglobin. Haemoglobin has two alpha chains and two beta chains, and four haems.

Dimers are proteins with two polypeptide subunits. Tetramers have four such units. In some cases the units are the same, but they may be different. Haemoglobin is a tetramer with two alpha chains and two beta chains.

Proteins have characteristic properties based on their organizational structure. One example of this is the venom produced by a stingray or jellyfish. If you are ever stung by a stingray or jellyfish, you should seek medical attention. However, some of the effects of a venom/toxin may be neutralized by immersing the cleaned wound in fresh, hot water. This will denature or change the organizational structure of the venom, resulting in a change in the properties of the protein, and a lessening of the symptoms.



The following table summarizes the four organizational structures of proteins.

Table 7.6 The organizational structure of proteins

Organizational structure	Cause
Primary	A sequence of 20 possible amino acids to produce a polypeptide chain. The amino acids are connected by peptide bonds
Secondary	Folding of the polypeptide chain into an α-helix or a β-pleated sheet. This is formed by hydrogen bonds between NH and CO groups
Tertiary	The secondary structure folded into a complex shape as a result of disulfide bridges, weak hydrogen and ionic bonds, and hydrophobic interactions
Quaternary	Not always present. Caused by the combination of groups of polypeptide chains, such as occurs in haemoglobin. All the bonds mentioned above are involved when this structure is present

Fibrous and globular proteins

Fibrous proteins are composed of many polypeptide chains in a long, narrow shape. They are usually insoluble in water. One example is collagen, which plays a structural role in the connective tissue of humans. Actin is another example, mentioned in Table 7.5. It is a major component of human muscle and is involved in contraction.

Globular proteins are more three-dimensional in their shape and are mostly water soluble. Haemoglobin, which delivers oxygen to body tissues, is one type of globular protein. Insulin is another globular protein; it is involved in regulating blood glucose levels in humans.

Polar and non-polar amino acids

Amino acids are often grouped based on the properties of their side chains (R-groups). Amino acids with non-polar side chains are hydrophobic. Non-polar amino acids are found in the regions of proteins that are linked to the hydrophobic area of the cell membrane.

Polar amino acids have hydrophilic properties, and they are found in regions of proteins that are exposed to water. Membrane proteins include polar amino acids towards the interior and exterior of the membrane. These amino acids create hydrophilic channels in proteins through which polar substances can move.

Polar and non-polar amino acids are important in determining the specificity of an enzyme. Each enzyme has a region called the active site. Only specific substrates can combine with particular active sites. Combination is possible when fitting occurs. Fitting involves the general shapes and polar properties of the substrate and of the amino acids exposed at the active site.

Exercises

11 Describe the functions of the three types of RNA involved in the translation process.

12 Explain the value of polysomes to a cell and an organism.

13 Draw a two-dimensional representation of a tRNA, labelling the 3′ and 5′ ends, the anticodon, and the point of attachment.

14 Explain why the primary level of protein organization determines the other levels.

15 What is the heme group containing iron called in the conjugated protein haemoglobin?

16 From the following DNA sequence, determine the sequence of amino acids that would be assembled.
T A C C G T G C A T A G A A A A T C

The polar properties of proteins are important for the properties of cell membranes. Ion channels in the cell membrane are produced from integral, polar proteins that allow the passage of small, charged particles through the hydrophobic region of the membrane. One type of ion channel allows the passage of calcium ions into muscle cells, thus initiating the process that brings about muscle contraction in the human body.

Polar amino acids include serine, threonine, tyrosine, and glutamine. All of these have a group with an electrical charge in their side chain. Non-polar amino acids have no electrical charges in their side groups. Examples of non-polar amino acids are tryptophan, leucine, alanine, and glycine.

To learn more about amino acids and protein structure, go to the hotlinks site, search for the title or ISBN, and click on Chapter 7: Section 7.3.

Practice questions

1 What does a nucleosome consist of ?

A DNA and histones.
B DNA and chromatin.
C Chromatin and nucleotides.
D Mature RNA and histones.

(*Total 1 mark*)

2 What are Okazaki fragments?

A Short lengths of RNA primase attached to the DNA during replication.
B Short sections of DNA formed during DNA replication.
C Nucleotides added by DNA polymerase I in the same direction as the replication fork.
D Sections of RNA removed by DNA polymerase III and replaced with DNA.

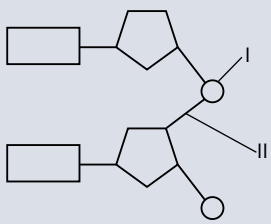
(*Total 1 mark*)

3 The sequence of nucleotides in a section of RNA is: GCCAUACGAUCG
What is the base sequence of the DNA sense strand?

A CGGUAUGCUAGC
B GCCATACGATCG
C CGGTATGCTAGC
D GCCAUACGAUCG

(*Total 1 mark*)

4 The diagram below shows two nucleotides linked together to form a dinucleotide.



(a) (i) Identify the chemical group labelled I. (1)

(ii) State the type of bond labelled II. (1)

(b) Distinguish between the sense and antisense strands of DNA during transcription. (1)

(c) Compare the DNA found in prokaryotic cells and eukaryotic cells. (2)

(*Total 5 marks*)

5 What is removed during the formation of mature RNA in eukaryotes?

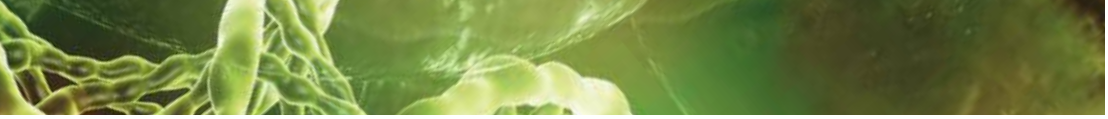
A Exons.
B Introns.
C Codons.
D Nucleosomes.

(*Total 1 mark*)

6 What does the universal nature of the genetic code allow?

A Change of genetic code in the same species.
B Transfer of genes between species.
C Formation of clones.
D Infection by bacteria.

(*Total 1 mark*)



7 What is a polysome?

A A ribosome that is synthesizing proteins from several mRNA molecules at the same time.

B A ribosome that is synthesizing different proteins for secretion.

C Several ribosomes using a mRNA molecule to synthesize protein at the same time.

D Several ribosomes that are synthesizing different proteins for use in the cytoplasm.

(*Total 1 mark*)

8 Up to two additional marks are available for the construction of your answers. (2)

(a) State four functions of proteins, giving a named example of each. (4)

(b) Outline the structure of ribosomes. (6)

(c) Explain the process of transcription leading to the formation of mRNA. (8)

(*Total 20 marks*)

9 The table below shows the codons that determine different amino acids in protein translation.

First base	Second base in codon				Third base
in codon	U	C	A	G	in codon
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	—	—	A
	Leu	Ser	—	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

What is the sequence of the amino acids that is being translated from the following mRNA sequence?

5′ AUGGGUGCUUAUUGGUAA 3′

A Met-Pro-Arg-Ile-Thr

B Met-Cys-Ser-Tyr-Trp

C Met-Gly-Ala-Tyr-Trp

D Met-Gly-Tyr-Ala-Thr

(*Total 1 mark*)

08 Metabolism, cell respiration, and photosynthesis

Essential ideas

8.1 Metabolic reactions are regulated in response to the cell's needs.

8.2 Energy is converted to a usable form in cell respiration.

8.3 Light energy is converted into chemical energy.

Organisms on our planet are part of a balanced system in which the products of the metabolic processes of one group of organisms are shared by all organisms. There is a constant interaction amongst all species. We must be ever-mindful of this balance and must continually work towards its maintenance.

Energy is a topic of discussion every day in our modern world. We talk about the energy needed to run our modes of transport. We talk about being so tired after a long day at school that we need a short nap. The need for food becomes essential at times to regain the energy levels necessary for us to function. This chapter will look at energy in living systems. The harnessing of energy by plants will be discussed in detail, as well as how both plants and animals may then release this harnessed energy in a form usable by the organism in question. The role of enzymes in these energy processes will be examined first.

8.1 Metabolism

Understandings:

- Metabolic pathways consist of chains and cycles of enzyme-catalysed reactions.
- Enzymes lower the activation energy of the chemical reactions that they catalyse.
- Enzyme inhibitors can be competitive or non-competitive.
- Metabolic pathways can be controlled by end-product inhibition.

Applications and skills:

- Application: End-product inhibition of the pathway that converts threonine to isoleucine.
- Application: Use of databases to identify potential new anti-malarial drugs.
- Skill: Calculating and plotting rates of reactions from raw experimental results.
- Skill: Distinguishing different types of inhibition from graphs at a specified substrate concentration.

Guidance

- *Enzyme inhibition should be studied using one specific example for competitive and non-competitive inhibition.*

NATURE OF SCIENCE

Developments in scientific research follow improvements in computing: developments in bioinformatics, such as the interrogation of databases, have facilitated research into metabolic pathways.

Metabolism

Your metabolism is the sum of all the chemical reactions that occur within you as a living organism. The type of reaction that uses energy to build complex organic molecules from simpler ones is called anabolism. The type of reaction that breaks down complex organic molecules with the release of energy is called catabolism. Table 8.1 summarizes anabolic and catabolic reactions.

Endergonic reactions are said to occur when the products of a chemical reaction have more energy than the reactants or substrates of the reaction. Endergonic reactions tend to occur in biosynthetic reactions in which more complex molecules are produced in the reaction. In contrast, exergonic reactions occur when the products of a chemical reaction have less energy than the reaction's reactants or substrates. Exergonic reactions tend to occur in degradative reactions or those reactions in which a complex molecule is broken down into simpler materials.

Table 8.1 Anabolic and catabolic reactions

Anabolic reactions	Catabolic reactions
Build complex molecules	Break down complex molecules
Are endergonic	Are exergonic
Are biosynthetic	Are degradative
Example: photosynthesis	Example: cellular respiration

Metabolic pathways

Almost all metabolic reactions in organisms are catalysed by enzymes. Many of these reactions occur in specific sequences and are called metabolic or biochemical pathways. A very simple, generalized, metabolic pathway is represented in Figure 8.1.

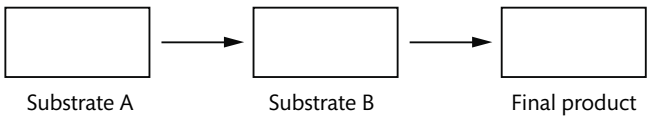


Figure 8.1 An example of a metabolic pathway.

Each arrow represents a specific enzyme that causes one substrate to be changed to another, until the final product of the pathway is formed. Some metabolic pathways consist of cycles of reactions instead of chains of reactions. Others involve both cycles and chains of reactions. Cell respiration and photosynthesis were discussed in Chapter 2, and both are complex pathways with chains and cycles of reactions. Metabolic pathways are usually carried out in designated compartments of the cell where the necessary enzymes are clustered and isolated. The enzymes required to catalyse every reaction in these pathways are determined by the cell's genetic makeup.

TOK

Biological processes and chemical pathways are usually quite complex. When scientists attempt to explain these complex reactions, they usually break them down into smaller, intermediate steps. These intermediate steps are then carefully researched and imitated. The hope is that eventually an understanding of the complete process is obtained. There are many subjects where knowledge is gained in a similar manner. Discuss some examples. Is this manner of understanding complex concepts always successful? What are some of the limitations of this approach?

Induced-fit model of enzyme action

Enzyme–substrate specificity was discussed in Section 2.5. Enzyme specificity is made possible by enzyme structure. Enzymes are very complex protein molecules with high molecular weights. The higher levels of protein structure allow enzymes to form unique areas, such as the active site. The active site is the region on the enzyme that binds to a particular substrate or substrates. This binding results in the reaction occurring much faster than would be expected without the enzyme.

In the 1890s Emil Fischer proposed the lock-and-key model of enzyme action. He suggested that substrate molecules fit like a key into a rigid section of the enzyme 'lock'. At the time this model provided a good explanation of the specificity of enzyme action. However, as knowledge about enzyme action has increased, Fischer's model has been modified.

Enzymes are globular proteins that, as a minimum, have the tertiary level of organization.

It is now obvious that many enzymes undergo significant changes in their conformation when substrates combine with their active site. The accepted new model for enzyme action is called the induced-fit model. A good way to visualize this model of enzyme action is to think of a hand and glove, the hand being the substrate and the glove being the enzyme. The glove looks a bit like the hand. However, when the hand is actually placed in the glove, there is an interaction that results in a conformational change of the glove, thus providing an induced fit.

The conformational changes and induced fit are the result of changes in the R-groups of the amino acids at the active site of the enzyme, as the enzyme interacts with the substrate or substrates.

In 1958, Daniel Koshland used a larger body of knowledge than had been available to Fischer, to present the induced-fit model.

Activation energy

When talking about enzyme action, we always refer to activation energy (AE). Activation energy is best understood as the energy necessary to destabilize the existing chemical bonds in the substrate of an enzyme–substrate catalysed reaction. Enzymes work by lowering the activation energy required (see Figure 8.2). This means that the enzymes cause chemical reactions to occur faster because they reduce the amount of energy needed to bring about the chemical reaction.

It is important to note that, even though enzymes lower the activation energy of a particular reaction, they do not alter the proportion of reactants to products.

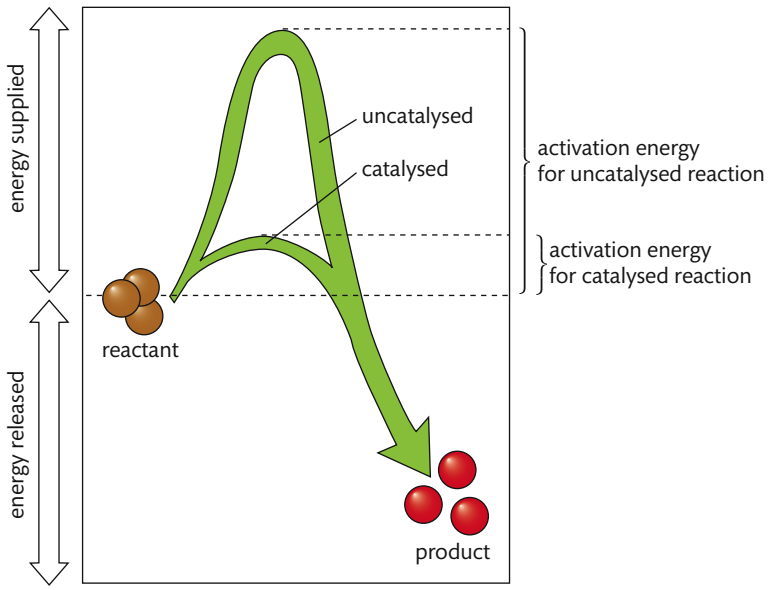


Figure 8.2 Enzymes accelerate reactions by lowering the activation energy required. This figure represents the effect of an enzyme on an exergonic reaction. The activation energy is needed to destabilize the chemical bonds in the reactant. The upper curve shows the activation energy when no enzyme is involved. The lower curve shows the activation energy required when an enzyme is present to catalyse the reaction.

Mechanism of enzyme action

The following summarizes the mechanism of enzyme action.

- The surface of the substrate contacts the active site of the enzyme.
- The enzyme changes shape to accommodate the substrate.
- A temporary complex called the enzyme–substrate complex forms.
- The activation energy is lowered and the substrate is altered by the rearrangement of the existing atoms.
- The transformed substrate, the product, is released from the active site.
- The unchanged enzyme is then free to combine with other substrate molecules.

Enzyme action can also be summarized by the following equation:

$$\mathbf{E + S \leftrightarrow ES \leftrightarrow E + P}$$

where E is the enzyme, S is the substrate, ES is the enzyme–substrate complex, and P is the product.

A coenzyme is not usually a protein. It has an essential role in the normal actions of an enzyme.

Inhibition

The effects of pH, temperature, and substrate concentration on the action of enzymes were discussed in Section 2.5. Here, we will discuss the effects of certain types of molecules on enzyme active sites. If a molecule affects an active site in some way, the activity of the enzyme may be altered.

Competitive inhibition

In competitive inhibition, a molecule called a competitive inhibitor competes directly with the usual substrate for the active site of an enzyme. The result is that the substrate will have fewer encounters with the active site and rate of the chemical reaction will be decreased. The competitive inhibitor must have a structure similar to the substrate to function in this way. An example is the use of sulfanilamide (a sulfa drug) to kill bacteria during an infection. Folic acid is essential to bacteria as a coenzyme. It is produced in bacterial cells by enzyme action on paraminobenzoic acid (PABA). The sulfanilamide competes with the PABA and blocks the enzyme. This prevents the production of folic acid resulting in the death of the bacteria. Because human cells do not use PABA to produce folic acid, they are unaffected by the drug.

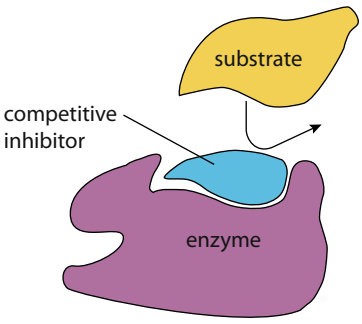


Figure 8.3 A competitive inhibitor blocks the active site of an enzyme so the substrate cannot bind to it.

Competitive inhibition may be reversible or irreversible. Reversible competitive inhibition may be overcome by increasing the substrate concentration. By doing this, there are more substrate molecules to bind with the active sites as they become available, and the chemical reaction may proceed more rapidly.

Non-competitive inhibition

Non-competitive inhibition involves an inhibitor that does not compete for the enzyme's active site. In this case, the inhibitor interacts with another site on the enzyme (see Figure 8.4). Non-competitive inhibition is also referred to as allosteric inhibition, and the site the inhibitor binds to is called the allosteric site. Binding at the allosteric site causes a change in the shape of the enzyme's active site, making it non-functional. Examples of non-competitive inhibition include metallic ions, such as mercury, binding to the sulfur groups of the component amino acids of many enzymes. This results in shape changes of the protein, which causes inhibition of the enzyme.

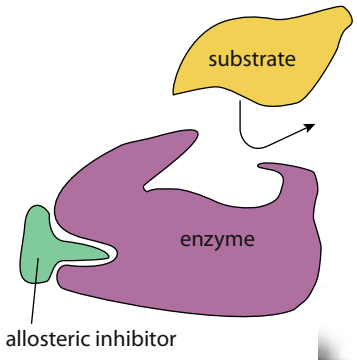


Figure 8.4 An allosteric (non-competitive) inhibitor combines with the allosteric site of an enzyme, causing the active site to change shape; the substrate cannot then bind to it.

Again, this type of inhibition may be reversible or irreversible. There are also examples of allosteric interactions activating an enzyme rather than inhibiting it.

End-product inhibition

End-product inhibition prevents the cell from wasting chemical resources and energy by making more of a substance than it needs. Many metabolic reactions occur in an assembly-line type of process so that a specific end product can be achieved. Each step of the assembly line is catalysed by a specific enzyme. When the end product is present in a sufficient quantity, the assembly line is shut down. This is usually done by inhibiting the action of the enzyme in the first step of the pathway. As the existing end product is used up by the cell, the first enzyme is reactivated. The enzyme that is inhibited and reactivated is an allosteric enzyme. When present in higher concentrations, the end product binds with the allosteric site of the first enzyme, thus bringing about inhibition. Lower concentrations of the end product result in fewer bindings with the allosteric site of the first enzyme, and, therefore, activation of the enzyme.

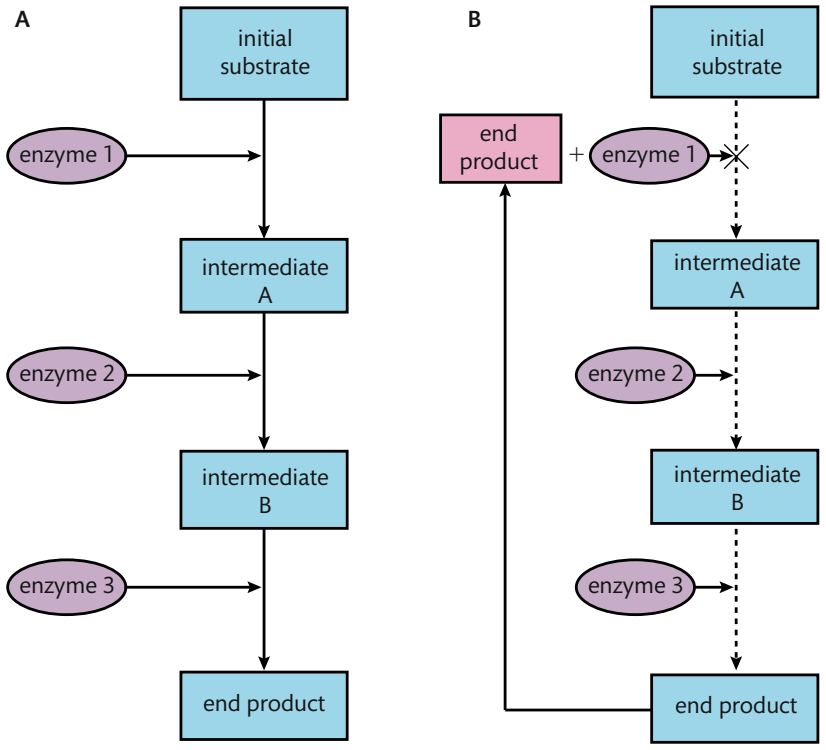


Figure 8.5 A short pathway of metabolic reactions with a specific end product that, when in sufficient quantity, causes end-product inhibition. This is also a form of negative feedback. The intermediates are essential molecules produced in the step-by-step pathway to achieve the end product. **A** represents a normal pathway with several enzymes producing intermediate compounds along the way. **B** represents feedback inhibition. In this condition a large amount of end-product is present. The end-product inhibits enzyme 1 in the pathway. The result is that the pathway is halted.

The bacterium *Escherichia coli* uses a metabolic pathway to produce the amino acid isoleucine from threonine. It is a five-step process. If isoleucine is added to the growth medium of *E. coli*, it inhibits the first enzyme in the pathway and isoleucine will not be synthesized. This situation will exist until the isoleucine is used up.

The inhibition of the first enzyme in the pathway prevents the build-up of intermediates in the cell. This is a form of negative feedback.

Many examples of enzyme inhibitors exist in medicine. Two examples of competitive inhibitors are ethanol and fomepizole. Either one of these two may be used as an antidote for ethylene glycol or methanol poisoning. Ethylene glycol and methanol may be used in producing car antifreeze, de-icing solutions, solvents, and cleaners. Fomepizole is a competitive inhibitor of alcohol dehydrogenase, which catalyses the breakdown of ethylene glycol and methanol into toxic metabolites. This allows other catalytic pathways to be activated, which do not result in toxic substances.

Worked example

Competitive and non-competitive inhibitors are examples of reversible enzyme inhibitors. When graphs of their effects are produced, certain characteristics can be seen. When a chemical is a competitive inhibitor, it competes for the active site of an enzyme, and its concentration must be kept high to keep the chemical reaction occurring at a slower rate. Non-competitive inhibitors do not compete for the active site of the enzyme. The result of this is that the rate of reaction will only increase if the enzyme concentration is increased. Examine Figure 8.6.

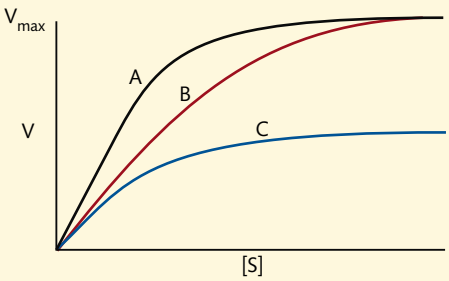


Figure 8.6 Enzyme inhibition. [S] = substrate concentration; V = reaction rate; V_{max} = maximum reaction rate.

Curve A represents a chemical reaction catalysed by an enzyme without the effect of an inhibitor. Curves B and C represent chemical reactions catalysed by enzymes affected by inhibitors.

1 Which curve represents the reaction in which a competitive inhibitor is active?
2 Which curve represents the effects of a non-competitive inhibitor?
3 Explain your answers.

Solutions

1 Curve B.
2 Curve C.
3 Curve C shows the action of a non-competitive inhibitor because it results in a lower maximum reaction rate. This occurs because the inhibitor binds to the enzyme present and is not released. The reaction rate will not increase as the substrate increases because there is a limited amount of enzymes still active. Curve B represents competitive inhibition because, as the substrate increases, the rate of the reaction also increases. This is because of the larger concentration of substrate out-competing the inhibitor for the active site of the enzyme. Curve B will eventually equal the maximum reaction when enough substrate is added.

When asked to differentiate between competitive and non-competitive inhibition curves on a graph, look to see if V_{max} is achieved as the substrate is increased. If it is, then competitive inhibition is being represented. If it is not achieved and is significantly less, non-competitive inhibition is being represented.

NATURE OF SCIENCE

Developments in areas such as bioinformatics have enhanced the research into metabolic pathways. Bioinformatics uses many areas of computer science and mathematics to look for unique events and patterns. This research uses large amounts of data stored in databases. Scientists often develop software to help this type of research. In 2011, an international team from the Genomics Institute of the Novartis Research Foundation and The Scripps Research Institution, by studying databases, discovered a new group of compounds that may lead to a new generation of anti-malarial drugs capable of both preventing the disease and of alleviating symptoms when the disease is already present in an individual.

This discovery came after mining the data for groups of related compounds that showed activity in the liver. They found a cluster of compounds related to imidazolopiperazine that is showing great promise.

To learn more about how enzymes work, go to the hotlinks site, search for the title or ISBN, and click on Chapter 8: Section 8.1.

Exercises

1 Explain why enzymes only work with specific substrates.
2 What determines whether an enzyme is competitively or non-competitively inhibited?
3 Where would the most efficient control of a metabolic pathway involving end-product inhibition occur?

8.2 Cell respiration

Understandings:

- Cell respiration involves the oxidation and reduction of electron carriers.
- Phosphorylation of molecules makes them less stable.
- In glycolysis, glucose is converted to pyruvate in the cytoplasm.
- Glycolysis gives a small net gain of ATP without the use of oxygen.
- In aerobic cell respiration pyruvate is decarboxylated and oxidized, and converted into acetyl compound and attached to coenzyme A to form acetyl coenzyme A in the link reaction.
- In the Krebs cycle, the oxidation of acetyl groups is coupled to the reduction of hydrogen carriers, liberating carbon dioxide.
- Energy released by oxidation reactions is carried to the cristae of the mitochondria by reduced NAD and FAD.
- Transfer of electrons between carriers in the electron transport chain in the membrane of the cristae is coupled to proton pumping.
- In chemiosmosis protons diffuse through ATP synthase to generate ATP.
- Oxygen is needed to bind with the free protons to form water to maintain the hydrogen gradient, resulting in the formation of water.
- The structure of the mitochondrion is adapted to the function it performs.

Applications and skills:

- Application: Electron tomography used to produce images of active mitochondria.
- Skill: Analysis of diagrams of the pathways of aerobic respiration to deduce where decarboxylation and oxidation reactions occur.
- Skill: Annotation of a diagram of a mitochondrion to indicate the adaptations to its functions.

Guidance

- *The names of the intermediate compounds in glycolysis and the Krebs cycle are not required.*

NATURE OF SCIENCE

Paradigm shift: the chemiosmotic theory led to a paradigm shift in the field of bioenergetics.

Oxidation and reduction

In Chapter 2 the general processes of respiration and photosynthesis were discussed. In this chapter we will consider these aspects of cellular metabolism in detail. It is important to recall that metabolism is the sum total of all the chemical reactions carried out by an organism. These reactions involve:

- catabolic pathways
- anabolic pathways.

Catabolic pathways result in the breakdown of complex molecules into smaller molecules. Conversely, anabolic pathways result in the synthesis of more complex molecules from simpler ones. Cellular respiration is an example of a catabolic pathway. Photosynthesis is an example of an anabolic pathway. To understand these complex pathways, it is essential to understand two general types of chemical reactions: oxidation and reduction.

Oxidation and reduction can be compared using a table like Table 8.2.

Table 8.2 A comparison of oxidation and reduction

Oxidation	Reduction
Loss of electrons	Gain of electrons
Gain of oxygen	Loss of oxygen
Loss of hydrogen	Gain of hydrogen
Results in many C–O bonds	Results in many C–H bonds
Results in a compound with lower potential energy	Results in a compound with higher potential energy

If asked in an exam to compare oxidation and reduction, using a table like Table 8.2 is an excellent way to structure the answer.

A useful way to remember the general meaning of oxidation and reduction is to think of the words 'oil rig'.

- oil = oxidation is loss (of electrons)
- rig = reduction is gain (of electrons).

These two reactions occur together during chemical reactions. Think of it in this way: one compound's or element's loss is another compound's or element's gain. This is shown by the following equation:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{energy}$$

Benedict's reagent is a chemical reagent commonly used to detect the presence of simple reducing sugars. It contains soluble blue copper (II) ions that may be reduced to copper (I) ions. These copper (I) ions are not soluble in water and will form a red–orange coloured precipitate. The colour of the precipitate indicates the quantity of simple sugar present: a green colour indicates a low sugar concentration and a red colour indicates a high sugar concentration. The electrons to reduce the Benedict's reagent resulting in the colour change come from the oxidation of the sugar molecules.

In this equation, glucose is oxidized because electrons are transferred from it to oxygen. The protons follow the electrons to produce water. The oxygen atoms that occur in the oxygen molecules on the reactant side of the equation are reduced. Because of this reaction, there is a large drop in the potential energy of the compounds on the product side of the equation.

Because oxidation and reduction always occur together, these chemical reactions are referred to as redox reactions. When redox reactions take place, the reduced form of a molecule always has more potential energy that the oxidized form of the molecule. Redox reactions play a key role in the flow of energy through living systems. This is because the electrons that are flowing from one molecule to the next are carrying energy with them. In a similar sort of way, the catabolic and anabolic pathways mentioned earlier are also closely associated with one another. You will see this association as you work through this chapter.

An overview of respiration

Section 2.8 provided an introduction to the process of cellular respiration. Three aspects of cellular respiration were discussed:

- glycolysis
- anaerobic respiration
- aerobic respiration.

As you will recall, glycolysis occurs in the cytoplasm of the cell, produces small amounts of adenosine triphosphate (ATP) and ends with the product known as pyruvate. If no oxygen is available, the pyruvate enters into anaerobic respiration. This occurs in the cytoplasm and it does not result in any further production of ATP. The products of anaerobic respiration are lactate or ethanol and carbon dioxide. If oxygen is available, the pyruvate enters aerobic respiration in the mitochondria of the cell. This process results in the production of a large number of ATPs, carbon dioxide and water.

In this section, we will discuss the cellular respiration that involves glycolysis and the three stages of aerobic respiration:

- the link reaction
- the Krebs cycle
- oxidative phosphorylation.

Glycolysis

The word glycolysis means 'sugar splitting' and this pathway is thought to be one of the first biochemical pathways to evolve. It uses no oxygen and occurs in the cytosol of the cell. No organelles are required. The sugar splitting proceeds efficiently in aerobic and anaerobic environments. Glycolysis occurs in both prokaryotic and eukaryotic cells. A hexose, usually glucose, is split in the process. This splitting actually involves many steps but we can explain it effectively in three stages.

1 Two molecules of ATP are used to begin glycolysis. In the first reaction, the phosphates from the ATPs are added to glucose to form fructose-1,6-biphosphate, a process called phosphorylation. The importance of phosphorylation in this step is that it creates a less stable molecule.

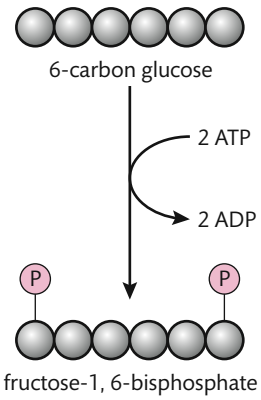


Figure 8.7 The first stage of glycolysis; the circles represent carbon atoms.

2 The less stable 6-carbon phosphorylated fructose is split into two 3-carbon sugars called glyceraldehyde-3-phosphate (G3P). This splitting process is known as lysis.

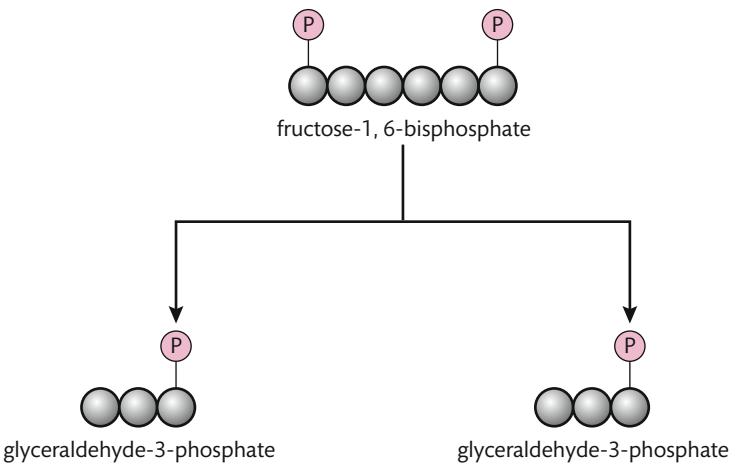


Figure 8.8 The second stage of glycolysis.

3 Once the two G3P molecules are formed, they enter an oxidation phase involving ATP formation and the production of the reduced coenzyme NAD. Each G3P or triose phosphate molecule undergoes oxidation to form a reduced molecule of NAD^+, which is NADH. As NADH is being formed, released energy is used to add an inorganic phosphate to the remaining 3-carbon compound. This results in a compound with two phosphate groups. Enzymes then remove the phosphate groups so that they can be added to adenosine diphosphate (ADP) to produce ATP. The end result is the formation of four molecules of ATP, two molecules of NADP, and two molecules of pyruvate. Pyruvate is the ionized form of pyruvic acid.

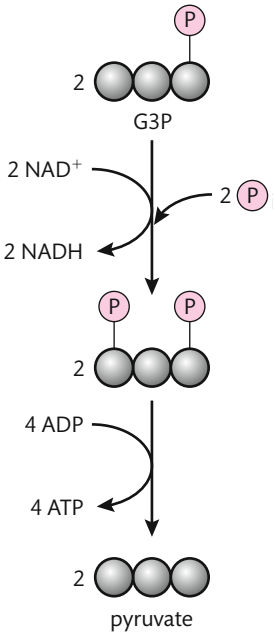


Figure 8.9 The third stage of glycolysis.

Once pyruvate is obtained, the next pathway is determined by the presence of oxygen. If oxygen is present, pyruvate enters the mitochondria and aerobic respiration occurs. If oxygen is not present, anaerobic respiration occurs in the cytoplasm. In the latter case, pyruvate is converted to lactase in animals, and ethanol and carbon dioxide in plants.

The way of producing ATP in glycolysis is called substrate-level phosphorylation because the phosphate group is transferred directly to ADP from the original phosphate-bearing molecule.

Summary of glycolysis

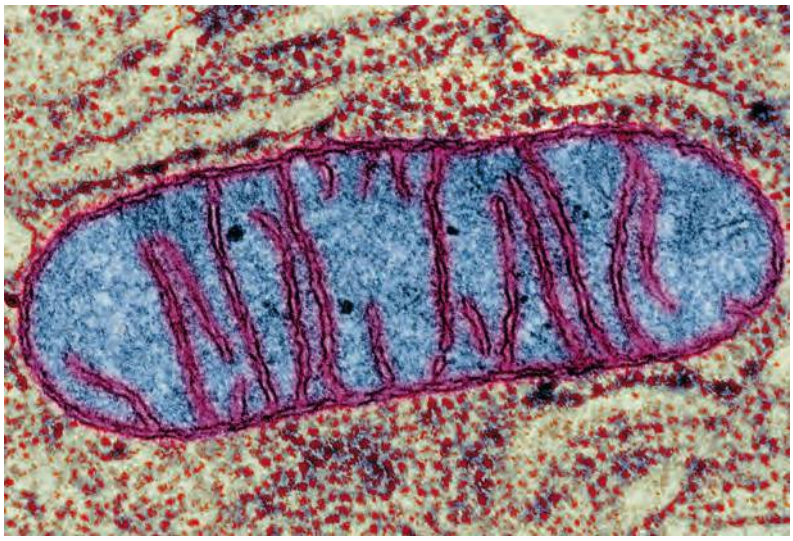
- Two ATPs are used to start the process.
- A total of four ATPs are produced: a net gain of two ATPs.
- Two molecules of NADH are produced.
- The pathway involves substrate-level phosphorylation, lysis, oxidation, and ATP formation.
- The pathway occurs in the cytoplasm of the cell.
- This metabolic pathway is controlled by enzymes. Whenever ATP levels in the cell are high, feedback inhibition will block the first enzyme of the pathway. This will slow or stop the process.
- Two pyruvate molecules are present at the end of the pathway.

To learn more about mitochondria, go to the hotlinks site, search for the title or ISBN, and click on Chapter 8: Section 8.2.

Mitochondria

It is inside the mitochondria and in the presence of oxygen that the remainder of cellular respiration occurs.

We discussed the structure of the mitochondrion in Chapter 1. You might like to refresh your memory of this because, as we discuss aerobic respiration, which occurs in the mitochondrion, we will refer to parts of this organelle.



This false-colour transmission electron micrograph (TEM) of a mitochondrion shows the internal structure. The matrix (blue) is permeated by the membranous cristae (pink).

The link reaction and the Krebs cycle

Once glycolysis has occurred and there is oxygen present, pyruvate enters the matrix of the mitochondria via active transport. Inside, pyruvate is decarboxylated, a reaction involving the loss of a carbon in the form of carbon dioxide, to form the 2-carbon acetyl group. This is the link reaction shown in Figure 8.10. The removed carbon is released as carbon dioxide, a waste gas. The acetyl group is then oxidized with the formation of reduced NAD^+. Finally, the acetyl group combines with coenzyme A (CoA) to form acetyl CoA.

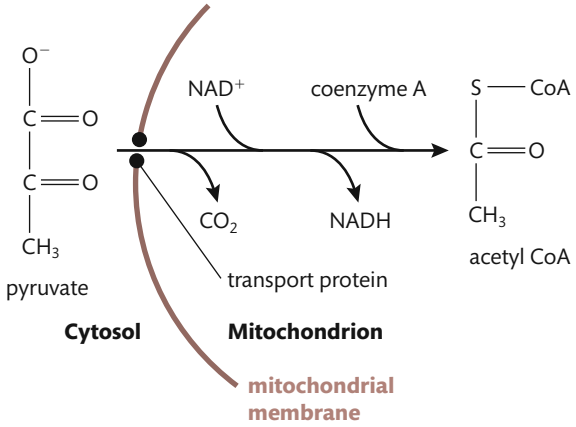


Figure 8.10 The link reaction.

Decarboxylation is the removal of a carbon atom.

A coenzyme is a molecule that aids an enzyme in its action. Coenzymes usually act as electron donors or acceptors.

The link reaction is controlled by a system of enzymes. The greatest significance of this reaction is that it produces acetyl CoA. Acetyl CoA may then enter the Krebs cycle to continue the aerobic respiration process.

So far in this discussion, the respiratory substrate has been a hexose. However, in reality, acetyl CoA can be produced from most carbohydrates and fats. Acetyl CoA can be synthesized into a lipid for storage purposes. This occurs when ATP levels in the cell are high.

If cellular ATP levels are low, the acetyl CoA enters the Krebs cycle. This cycle is also called the tricarboxylic acid cycle. It occurs in the matrix of the mitochondrion and is referred to as a cycle because it begins and ends with the same substance. This is a

characteristic of all cyclical pathways in metabolism. You do not need to remember the names of all the compounds formed in the Krebs cycle. However, it is important that you understand the overall process.

Let's consider the cycle as a series of steps.

1 Acetyl CoA from the link reaction combines with a 4-carbon compound called oxaloacetate. The result is a 6-carbon compound called citrate.

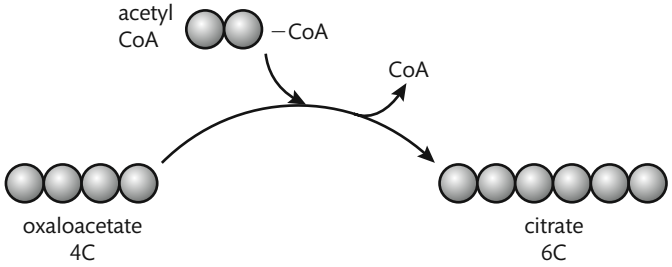


Figure 8.11 Acetyl CoA combines with oxaloacetate to form citrate.

2 Citrate (a 6-carbon compound) is oxidized to form a 5-carbon compound. In this process, the carbon is released from the cell (after combining with oxygen) as carbon dioxide. While the 6-carbon compound is oxidized, NAD^+ is reduced to form NADH.

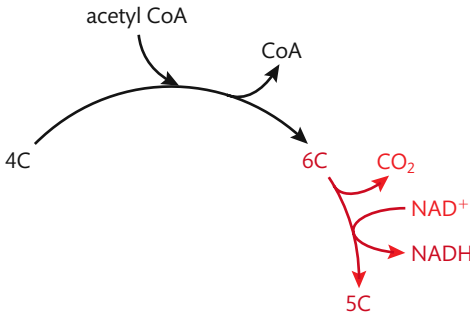


Figure 8.12 Then a 5-carbon compound is formed.

3 The 5-carbon compound is oxidized and decarboxylated to form a 4-carbon compound. Again, the removed carbon combines with oxygen and is released as carbon dioxide. Another NAD^+ is reduced to form NADH.

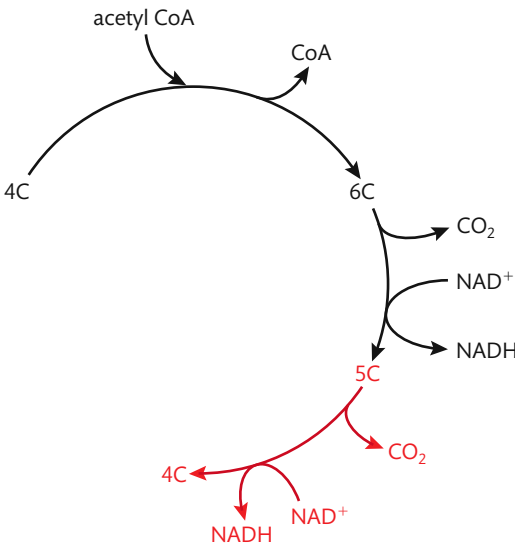


Figure 8.13 Next, a 4-carbon compound is produced.

4 The 4-carbon compound undergoes various changes resulting in several products. One product is another NADH. The coenzyme FAD is reduced to form $FADH_2$. There is also a reduction of an ADP to form ATP. The 4-carbon compound is changed during these steps to re-form the starting compound of the cycle, oxaloacetate. The oxaloacetate may then begin the cycle again.

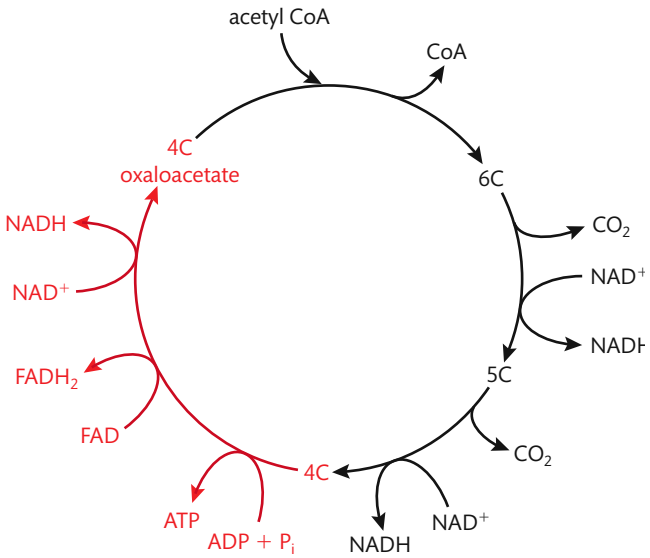


Figure 8.14 Finally, the 4-carbon compound is converted to oxaloacetate.

It is important to remember that the Krebs cycle will run twice for each glucose molecule entering cellular respiration. This is because a glucose molecule forms two pyruvate molecules. Each pyruvate produces one acetyl CoA that enters the cycle. Look again at the complete Krebs cycle (Figure 8.14) and note the following products that result from the breakdown of one glucose molecule:

- 2 ATP molecules per molecule of glucose
- 6 molecules of NADH (which allow energy storage and transfer)
- 2 molecules of $FADH_2$
- 4 molecules of carbon dioxide (released).

Two carbon dioxides are released for each glucose molecule during the link reaction. Four carbon dioxides are released during the Krebs cycle. This accounts for all six of the carbon atoms that were present in the initial glucose molecule. Glucose is completely catabolized and its original energy is now carried by NADH and $FADH_2$ or is in ATP.

So far, only four ATPs have been gained, six are generated (four from glycolysis and two from the Krebs cycle) but two are used to start the process of glycolysis. Each of these ATPs has been produced by substrate-level phosphorylation.

Ultimately, the breakdown of each glucose molecule results in a net gain of 36 ATPs. Let's now consider the phase of cellular respiration where most of the ATPs are produced. In this phase oxidative phosphorylation is the means by which the ATPs are produced.

CHALLENGE YOURSELF

1 Examine Figure 8.15.

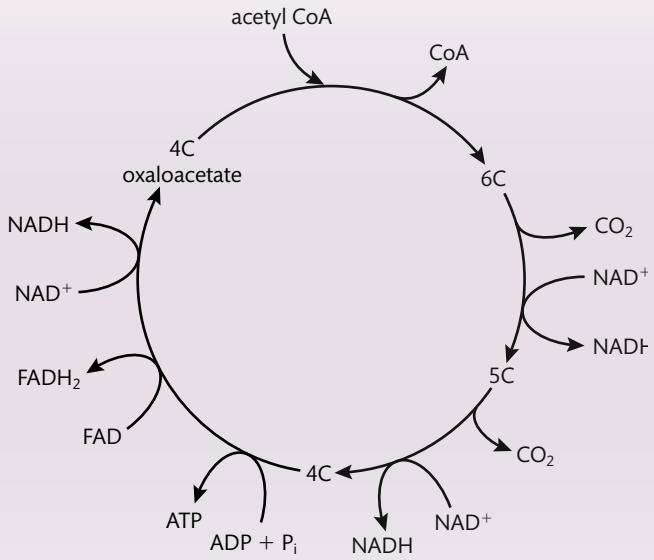


Figure 8.15

(a) Redraw the figure and place an arrow and the letter D at the two locations where decarboxylation occurs. On the same figure place an arrow and the letter O at the five locations where oxidation of a cyclic intermediate compound occurs.
(b) How did you determine the two locations where decarboxylation occurred?
(c) How did you determine the five locations where oxidation occurred?

To learn more about the electron transport chain, go to the hotlinks site, search for the title or ISBN, and click on Chapter 8: Section 8.2.

Electron transport chain and chemiosmosis

The electron transport chain is where most of the ATPs from glucose catabolism are produced. It is the first stage of cellular respiration where oxygen is actually needed, and it occurs within the mitochondrion. However, unlike the Krebs cycle, which occurred in the matrix, the electron transport chain occurs on the inner mitochondrial membrane and on the membranes of the cristae.

The haem group of the carrier is the part that is easily reduced and oxidized.

Embedded in the membranes involved are molecules that are easily reduced and oxidized. These carriers of electrons (energy) are close together and pass the electrons from one to another because of an energy gradient. Each carrier molecule has a slightly different electronegativity, and, therefore, a different attraction for electrons. Most of these carriers are proteins with haem groups and are referred to as cytochromes. One carrier is not a protein and is called coenzyme Q.

In this chain, electrons pass from one carrier to another because the receiving molecule has a higher electronegativity and, therefore, a stronger attraction for electrons. In the process of electron transport, small amounts of energy are released. The sources of the electrons that move down the electron transport chain are the coenzymes NADH and $FADH_2$ from the previous stages of cellular respiration.

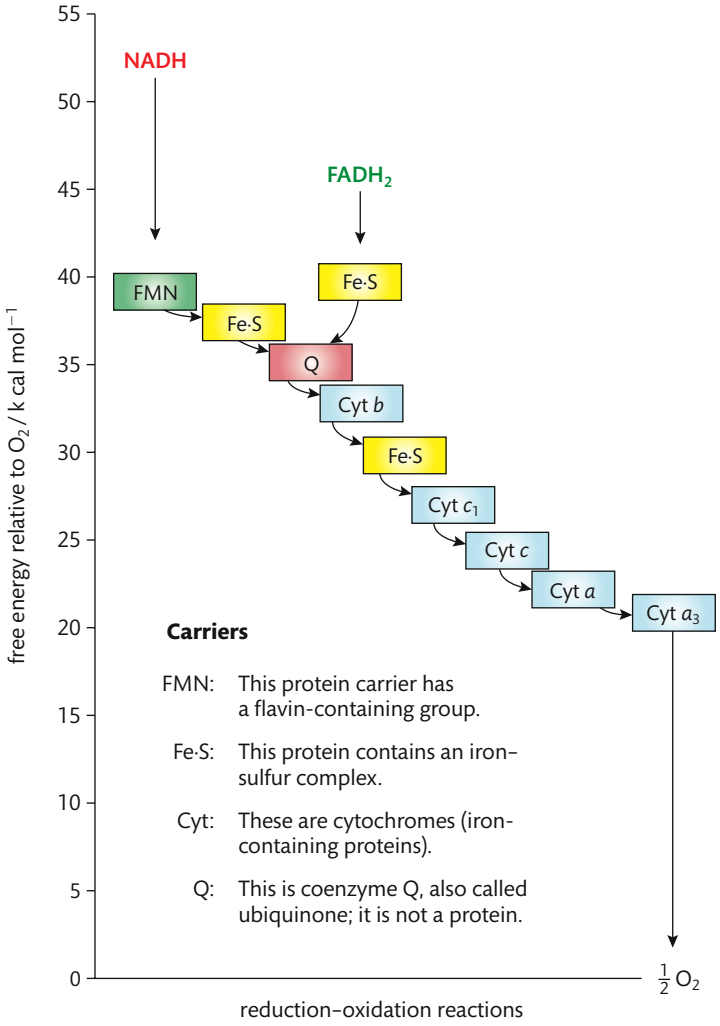


Figure 8.16 The oxidation–reduction reactions of the electron transport chain. It is not necessary for you to remember all the names of the carriers.

In Figure 8.16 it is clear that the electrons are stepping down in potential energy as they pass from one carrier to another. It is important to note that:

- $FADH_2$ enters the electron transport chain at a lower free energy level than NADH, thus $FADH_2$ allows the production of two ATPs while NADH allows the production of three ATPs
- at the very end of the chain, the de-energized electrons combine with available oxygen.

Oxygen is the final electron acceptor because it has a very high electronegativity and, therefore, a strong attraction for electrons. When the electrons combine with the oxygen, so do two hydrogen ions from the aqueous surroundings. The result is water. Because of the way this water is formed, it is referred to as water of metabolism.

It is also clear from Figure 8.16 that there is a fairly large number of electron carriers. Because of the larger number, the electronegativity difference between adjacent carriers is not as great. This means that lower amounts of energy are lost at each exchange. These lower amounts of energy are effectively harnessed by the cell to carry out phosphorylation. If the amount of energy lost at each exchange was high, much of it could not be used and the cell could even be damaged.

The kangaroo rat from a desert region of the USA gets 90% of its daily water intake from water of metabolism. In contrast, a typical human only gets 12% of his or her daily water intake from metabolism.

No ATPs are produced directly by the electron transport chain. However, this chain is essential to chemiosmosis, which does produce the ATP.

So energy is now available as a result of the electron transport chain. This is the energy that allows the addition of phosphate and energy to ADP to form ATP. The process by which this occurs is called chemiosmosis. Chemiosmosis involves the movement of protons (hydrogen ions) to provide energy so that phosphorylation can occur. Because this type of phosphorylation utilizes an electron transport chain, it is called oxidative phosphorylation. Substrate-level phosphorylation, mentioned in the earlier phases of cellular respiration, does not involve an electron transport chain.

Before continuing, it is essential to review the interior structure of the mitochondrion. In the process of cellular respiration, the structure of the mitochondrion is very closely linked to its function. The matrix is the area where the Krebs cycle occurs. The cristae provide a large surface area for the electron transport chain to function on. The membranes also provide a barrier, allowing proton accumulation on one side. Embedded in the membranes are the enzymes and other compounds necessary for the processes of the electron transport chain and chemiosmosis to occur.

Using any diagram or photomicrograph of a mitochondrion, practise annotating where the processes of aerobic respiration occur.

The inner membranes of the mitochondria have numerous copies of an enzyme called ATP synthase. This enzyme uses the energy of an ion gradient to allow the phosphorylation of ADP. The ion gradient is created by a hydrogen ion concentration difference that occurs across the cristae membranes. Figure 8.17 shows oxidative phosphorylation.

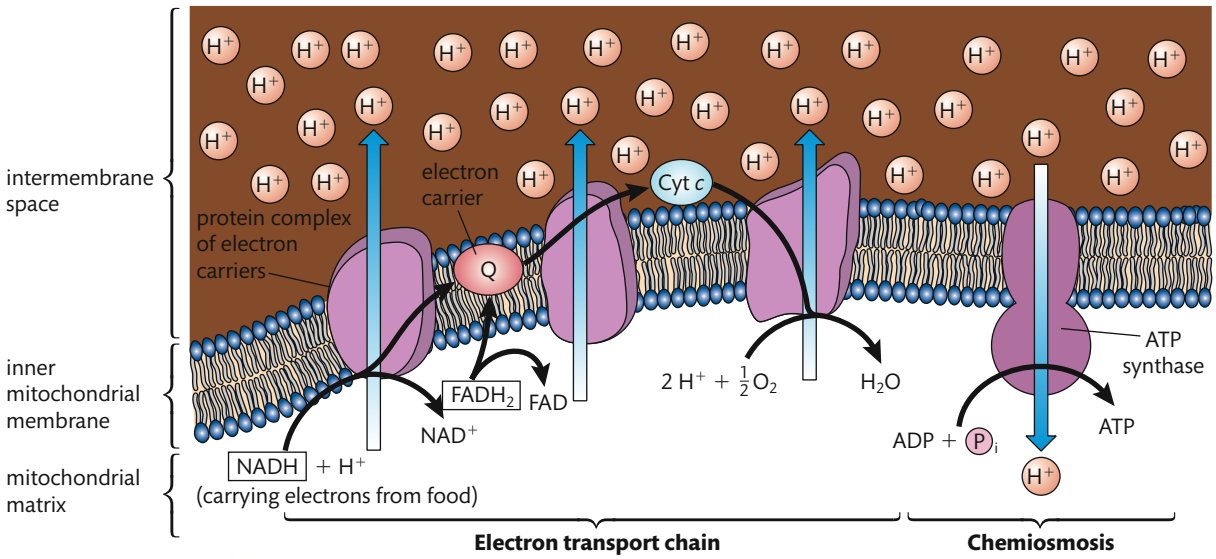


Figure 8.17 Oxidative phosphorylation occurs at the inner membranes of the mitochondria of a cell. The pumping actions of the carriers result in a high concentration of hydrogen ions in the intermembrane space. This accumulation allows movement of the hydrogen ions through the enzyme ATP synthase. The enzyme uses the energy from the hydrogen flow to couple phosphate with ADP to produce ATP.

In Figure 8.17, note the three labelled areas on the left: the intermembrane space, inner mitochondrial membrane, and mitochondrial matrix. Also, note that hydrogen ions are being pumped out of the matrix into the intermembrane space. The energy for this pumping action is provided by the electrons as they are de-energized moving through the electron transport chain. This creates the different hydrogen ion concentration on the two sides of the cristae membranes, mentioned above. With the higher hydrogen ion concentration in the intermembrane space, these ions begin to move passively through a channel in ATP synthase back into the mitochondrial matrix. As the hydrogen ions move through the ATP synthase channel, the enzyme harnesses the available energy, thus allowing the phosphorylation of ADP.

Unfortunately, since the beginning of scientific experimentation, there have been instances of improper presentation of results. These improper presentations have included improper data reporting or even data fabrication. In the scientific community, this misconduct is extremely frowned upon. Discuss the possible repercussions to science research as a whole when such misconduct occurs. Continue your discussion to include reasons why scientists sometimes present improper or fabricated data.

TOK

Because of the hydrophobic region of the membrane, the hydrogen ions can only pass through the ATP synthase channel. Some poisons that affect metabolism act by establishing alternative pathways through the membrane, thus preventing ATP production.

Summary of ATP production in cellular respiration

We have now described the complete catabolism of one molecule of glucose. The raw materials were glucose and oxygen. Many enzymes, carriers, and other molecules are involved in the process. The products are carbon dioxide, water, and ATP. The ATPs are essential because they provide the energy by which life is maintained. We can describe the energy flow in the general process as shown in Figure 8.18.

Glucose → NADH or $FADH_2$ → Electron transport chain → Chemiosmosis → ATP

Figure 8.18

To account for the production of ATP in cellular respiration, let's look at the three main processes, glycolysis, the Krebs cycle, and the electron transport chain, in a table.

Table 8.3 The processes of cellular respiration

Process	ATP used	ATP produced	Net ATP gain
Glycolysis	2	4	2
Krebs cycle	0	2	2
Electron transport chain and chemiosmosis	0	32	32
Total	2	38	36

Theoretically 36 ATPs are produced by cellular respiration, but in reality the number is closer to 30. This is thought to be because some hydrogen ions move back to the matrix without going through the ATP synthase channel. Also, some of the energy from hydrogen ion movement is used to transport pyruvate into the mitochondria. The 30 ATPs generated by cellular respiration account for approximately 30% of the energy present in the chemical bonds of glucose. The remainder of the energy is lost from the cell as heat.

A final look at respiration and the mitochondrion

Cellular respiration is the process by which ATP is provided to the organism so that it can live. It is a very complex series of chemical reactions, most of which occur in the mitochondrion. Let's end our discussion of this essential-to-life process by looking at a table showing the parts of the mitochondrion and how those parts allow cellular respiration.

Table 8.4 The role of the mitochondrion in cellular respiration

Feature	Role
Outer mitochondrial membrane	A membrane that separates the contents of the mitochondrion from the rest of the cell
Matrix	An internal cytosol-like area that contains the enzymes for the link reaction and the Krebs cycle
Cristae	Tubular regions surrounded by membranes that increase the surface area for oxidative phosphorylation
Inner mitochondrial membrane	A membrane that contains the carriers for the electron transport chain and ATP synthase for chemiosmosis
Space between inner and outer membranes	A reservoir for hydrogen ions (protons)

The overall equation for cellular respiration is:

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O + \text{energy (as heat or ATP)}$$

All organisms need the ability to produce ATP for energy, so all organisms carry out respiration.

CHALLENGE YOURSELF

2 Annotate the diagram of a mitochondrion provided in Figure 8.19 to indicate the adaptations that allow the mitochondrion to carry out its essential functions.

Figure 8.19

Exercises

4 Using ideal ATP production numbers, how many ATPs would an individual generate if he or she consumed only pyruvate and carried one pyruvate molecule through cellular respiration?

5 Striated or voluntary muscles that occur in humans generally have a larger number of mitochondria than other cell types. Why is this important?

6 If both NAD and FAD are reduced, which would allow the greater production of ATPs via the electron transport chain and chemiosmosis?

7 If an individual took a chemical that increased the ability of hydrogen ions to move through the phospholipid bilayer of the mitochondrial membranes, what would the effect be on ATP production?

8 If ATP synthase was not present in the cristae of a mitochondrion, what would be the effect?

NATURE OF SCIENCE

Developments in scientific research follow improvements in apparatus: sources of ^{14}C and autoradiography enabled Calvin to elucidate the pathways of carbon fixation.

To see an introduction to photosynthesis, go to the hotlinks site, search for the title or ISBN, and click on Chapter 8: Section 8.3.

8.3 Photosynthesis

Understandings:

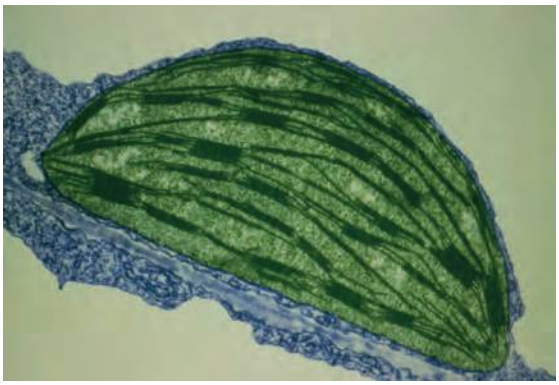
- Light-dependent reactions take place in the intermembrane space of the thylakoids.
- Light-independent reactions take place in the stroma.
- Reduced NADP and ATP are produced in the light-dependent reactions.
- Absorption of light by photosystems generates excited electrons.
- Photolysis of water generates electrons for use in the light-dependent reactions.
- Transfer of excited electrons occurs between carriers in thylakoid membranes.
- Excited electrons from Photosystem II are used to contribute to generate a proton gradient.
- ATP synthase in thylakoids generates ATP using the proton gradient.
- Excited electrons from Photosystem I are used to reduce NADP.
- In the light-independent reactions a carboxylase catalyses the carboxylation of ribulose bisphosphate.
- Glycerate 3-phosphate is reduced to triose phosphate using reduced NADP and ATP.
- Triose phosphate is used to regenerate RuBP and produce carbohydrates.
- Ribulose bisphosphate is reformed using ATP.
- The structure of the chloroplast is adapted to its function in photosynthesis.

Applications and skills:

- Application: Calvin's experiment to elucidate the carboxylation of RuBP.
- Skill: Annotation of a diagram to indicate the adaptations of a chloroplast to its function.

The chloroplast

Some people refer to the chloroplast as a photosynthetic machine. They are not wrong. Unlike respiration, where some of the steps occur outside the mitochondrion, all of the photosynthetic process occurs within the chloroplast. Chloroplasts, along with mitochondria, represent possible evidence for the theory of endosymbiosis, discussed in Chapter 1, Section 1.5. Both organelles have an extra outer membrane (indicating a need for protection in a potentially hostile environment), their own DNA, and they are very near in size to a typical prokaryotic cell.



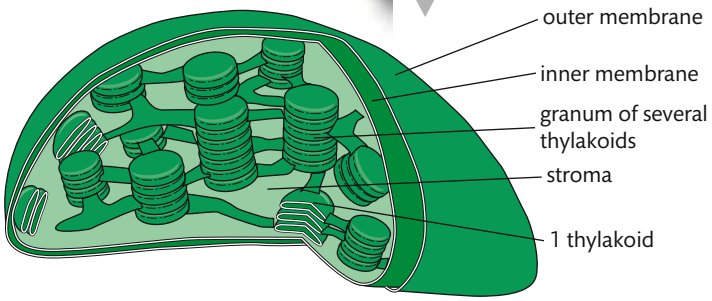


Figure 8.20 This false-colour TEM and drawing show the structure of a chloroplast. Can you find as many parts in the TEM as are labelled in the drawing?

The structure of the chloroplast was discussed in Chapter 1. You may want to return to that chapter for a brief refresher. Chloroplasts occur mostly within the cells of the photosynthetic factory of the plant, the leaves. However, some plants have chloroplasts in cells of other organs.

Plastids are a group of closely related organelles that occur in photosynthetic eukaryotic cells. There are three types of plastid that occur in plant cells:

- chloroplasts, which are green and involved in photosynthesis
- leucoplasts, which are white or 'clear' and function as energy storehouses
- chromoplasts, which are brightly coloured and synthesize and store large amounts of orange, red, or yellow pigments.

All these plastids develop from a common proplastid.

The overall process of photosynthesis

During the discussion on respiration, we considered the means by which the cell breaks down chemical bonds in glucose to produce ATP. In this section, the discussion centres on the establishment of chemical bonds to produce organic compounds. Using light energy, the raw materials of photosynthesis are carbon dioxide and water. Many enzymes are involved to enable the formation of products that include glucose, more water, and oxygen. The overall equation is:

$$6\,CO_2 + 12\,H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6\,H_2O + 6\,O_2$$

Water occurs on both sides because 12 molecules are consumed and 6 molecules are produced. Clearly, photosynthesis is essentially the reverse of respiration. Whereas respiration is, in general, a catabolic process, photosynthesis is, in general, an anabolic process. Photosynthesis occurs in organisms referred to as autotrophs. These organisms make their own food. Non-photosynthetic and non-chemosynthetic organisms are referred to as heterotrophs. They must obtain their food (which is necessary for energy) from other organisms.

Photosynthesis involves two major stages:

- the light-dependent reaction
- the light-independent reaction.

Light energy behaves as if it exists in discrete packets called photons. Shorter wavelengths of light have greater energy within their photons than longer wavelengths. Photons can transfer their energy upon interaction with other particles. This transfer of energy occurs many times in photosynthesis.

The light-dependent reaction

The light-dependent reaction occurs in the thylakoids or grana of the chloroplast. A stack of thylakoids make up a granum (plural grana). Light supplies the energy for this reaction to occur. The ultimate source of light is the Sun. Even though plants may survive quite well when they receive light from sources other than the Sun, most plants on our planet rely on the Sun for the energy necessary to drive photosynthesis.

To absorb light, plants have special molecules called pigments. There are several different pigments in plants, and each effectively absorbs photons of light at different wavelengths. The two major groups are the chlorophylls and the carotenoids. These pigments are organized on the membranes of the thylakoids. The regions of organization are called photosystems and include:

- chlorophyll *a* molecules
- accessory pigments
- a protein matrix.

The reaction centre is the portion of the photosystem that contains:

- a pair of chlorophyll *a* molecules
- a matrix of protein
- a primary electron acceptor.

Bacteria that carry out photosynthesis have only one type of photosystem. However, modern-day plants have two types of photosystem. Each absorbs light most efficiently at a different wavelength. Photosystem I is most efficient at 700 nanometres (nm) and is labelled as P700. Photosystem II is most efficient at 680 nm and is labelled as P680. These two photosystems work together to bring about a non-cyclical electron transfer. Figure 8.21 shows the overall light-dependent reaction of photosynthesis involving non-cyclic photophosphorylation (non-cyclic electron flow).

The numbered descriptions that follow refer to the numbered steps in Figure 8.21.

1. A photon of light is absorbed by a pigment in Photosystem II and is transferred to other pigment molecules until it reaches one of the chlorophyll *a* (P680) molecules in the reaction centre. The photon energy excites one of the chlorophyll *a* electrons to a higher energy state.
2. This electron is captured by the primary acceptor of the reaction centre.
3. Water is split by an enzyme to produce electrons, hydrogen ions, and an oxygen atom. This process is driven by the energy from light and is called photolysis. The electrons are supplied one by one to the chlorophyll *a* molecules of the reaction centre.

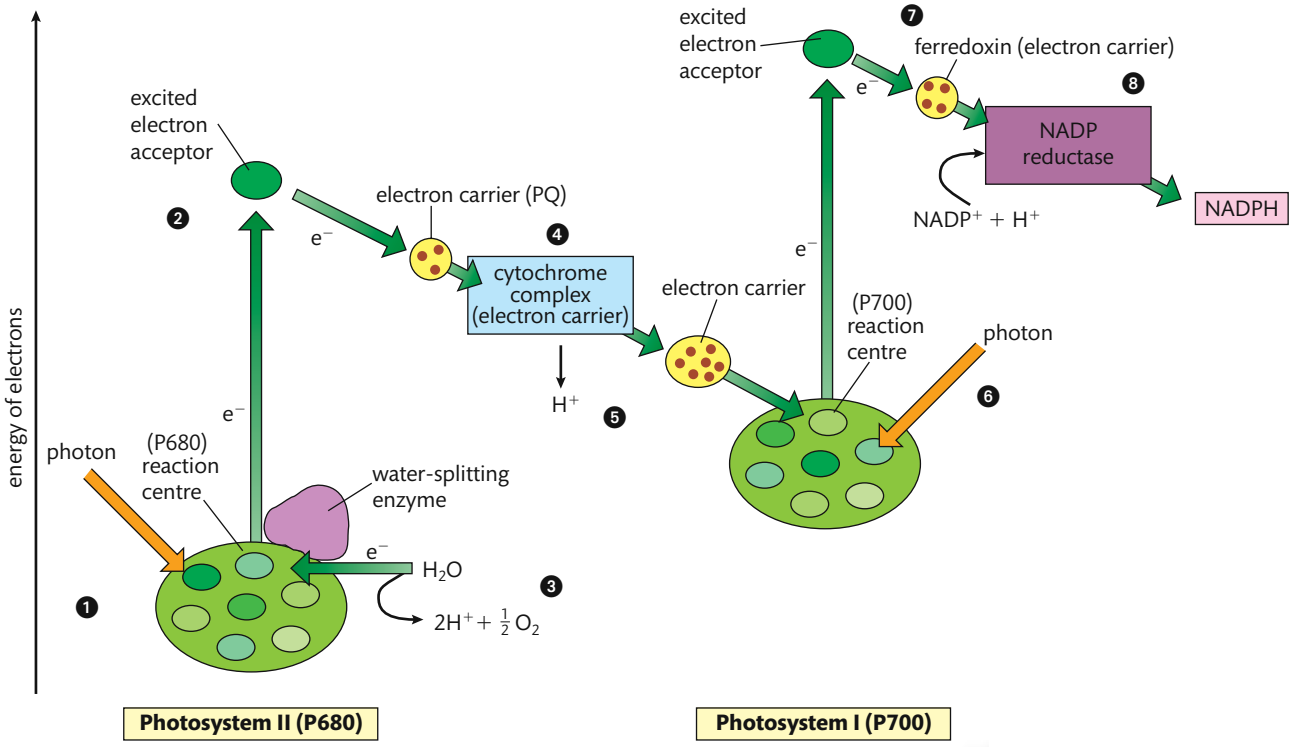


Figure 8.21 The light-dependent reaction of photosynthesis.

4 The excited electrons pass from the primary acceptor down an electron transport chain, losing energy at each exchange. The first of the three carriers is plastoquinone (PQ). The middle carrier is a cytochrome complex.

5 The energy lost from the electrons moving down the electron transport chain drives chemiosmosis (similar to that in respiration) to bring about phosphorylation of ADP to produce ATP.

6 A photon of light is absorbed by a pigment in Photosystem I. This energy is transferred through several accessory pigments until received by a chlorophyll *a* (P700) molecule. This results in an electron with a higher energy state being transferred to the primary electron acceptor. The de-energized electron from Photosystem II fills the void left by the newly energized electron.

7 The electron with the higher energy state is then passed down a second electron transport chain that involves the carrier ferredoxin.

8 The enzyme NADP reductase catalyses the transfer of the electron from ferredoxin to the energy carrier $NADP^+$. Two electrons are required to reduce $NADP^+$ fully to NADPH.

NADPH and ATP are the final products of the light-dependent reaction. They supply chemical energy for the light-independent reaction to occur. The explanation above also shows the origin of the oxygen released by photosynthesizing plants (step 3). However, you need to know more detail about the production of ATP.

ATP production in photosynthesis is very similar to ATP production in respiration. Chemiosmosis allows the process of phosphorylation of ADP. In this case, the energy to drive chemiosmosis comes from light. As a result, we refer to the production of ATP in photosynthesis as photophosphorylation.

Besides the non-cyclic electron pathway used to produce ATP by photophosphorylation, there is an alternative pathway involving a cyclic pathway. This cyclic pathway is discussed on the following page.

A comparison of chemiosmosis in respiration and photosynthesis is shown in Table 8.5.

Table 8.5 A comparison of chemiosmosis

Respiration chemiosmosis	Photosynthesis chemiosmosis
Involves an electron transport chain embedded in the membranes of the cristae	Involves an electron transport chain embedded in the membranes of the thylakoids
Energy is released when electrons are exchanged from one carrier to another	Energy is released when electrons are exchanged from one carrier to another
Released energy is used to pump hydrogen ions actively into the intermembrane space	Released energy is used to pump hydrogen ions actively into the thylakoid space
Hydrogen ions come from the matrix	Hydrogen ions come from the stroma
Hydrogen ions diffuse back into the matrix through the channels of ATP synthase	Hydrogen ions diffuse back into the stroma through the channels of ATP synthase
ATP synthase catalyses the phosphorylation of ADP to form ATP	ATP synthase catalyses the photophosphorylation of ADP to form ATP

In both cases ATP synthase is embedded along with the carriers of the electron transport chain in the membranes involved.

In photosynthesis, the production of ATP occurs between Photosystem II and Photosystem I. Study Figure 8.22. Notice that the b_6–f complex, which is a cytochrome complex, pumps the hydrogen ions into the thylakoid space. This increases the concentration of these ions, which then move passively through the ATP synthase channel, providing the energy to phosphorylate ADP.

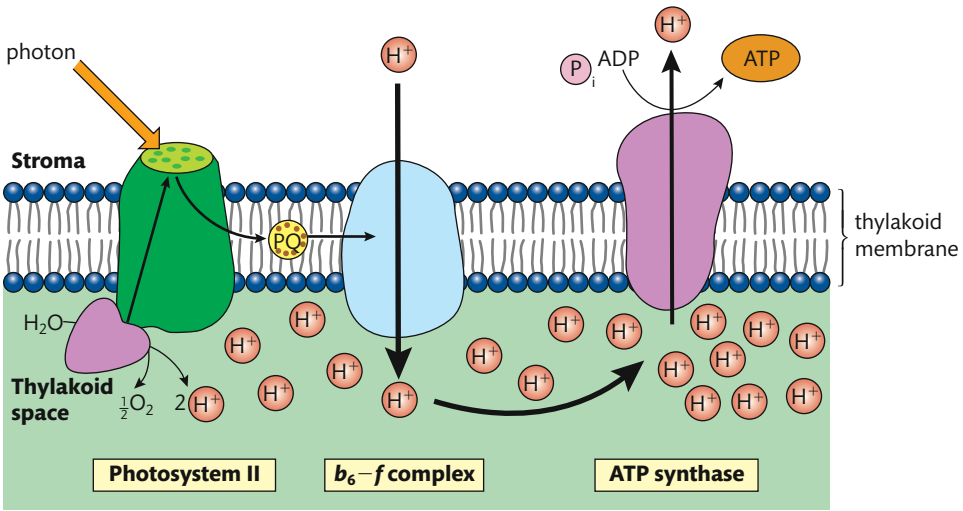


Figure 8.22 Chemiosmosis in a plant cell chloroplast.

The process just described is also known as non-cyclic photophosphorylation. There is another way the light-dependent reaction of photosynthesis may produce ATP. It is called cyclic photophosphorylation. It proceeds only when light is not a limiting factor and when there is an accumulation of NADPH in the chloroplast. In this process, light-energized electrons from Photosystem I flow back to the cytochrome complex of the electron transport chain between Photosystem II and Photosystem I (see Figure 8.21). From the cytochrome complex, the electrons move down the remaining electron transport chain allowing ATP production via chemiosmosis. These ATPs are then shuttled to the Calvin cycle so that it can proceed more rapidly.

Experiment to demonstrate electron transfer in chloroplasts

Safety alerts: Use safety goggles and lab aprons. Be cautious in the use of chemicals and glassware. Be careful of light sources as they may be hot. Dispose of chemicals as directed by your teacher. Wash your hands thoroughly upon completion of the activity.

Electrons energized by light allow the production of ATP and NADPH in the light-dependent reaction of photosynthesis. In this experiment, DCPIP or DPIP (2,6-dichlorophenol-indophenol) will be used to replace NADP in the light-dependent reaction. DCPIP is a blue colour, but turns colourless when reduced. There is a direct relationship between the rate of photosynthesis and the change in colour from blue DCPIP to its reduced, clear form.

Full details with a worksheet of how to carry out this experiment are available on your eBook.

Worksheets

Data and observations

Table 8.6

Experimental cuvette	Start colour/ absorbance	Finish colour/absorbance
Dark/unboiled		
Light/unboiled		
Light/boiled		

Questions

1. What was the control in this experiment?
2. What product would have received the energized electrons if DCPIP had not been added?
3. What is the actual source of the electrons that reduced the DCPIP?
4. What was the effect of darkness on the reduction of DCPIP? Explain.
5. What was the effect of boiling the chloroplasts on this experiment? Explain.

The light-independent reaction

The light-independent reaction occurs within the stroma or cytosol-like region of the chloroplast.

The ATP and NADPH produced by the light-dependent reaction provide the energy and reducing power for the light-independent reaction to occur. Up to this point there has been no mention of carbohydrate production. Therefore, as we know glucose is a product of photosynthesis, the result of the light-independent reaction must be the production of glucose.

The light-independent reaction involves the Calvin cycle (see Figure 8.23), which occurs in the stroma of the chloroplast. Because it is a cycle, it begins and ends with the same substance. You should recall that a similar cyclic metabolic pathway occurred in respiration: the Krebs cycle.

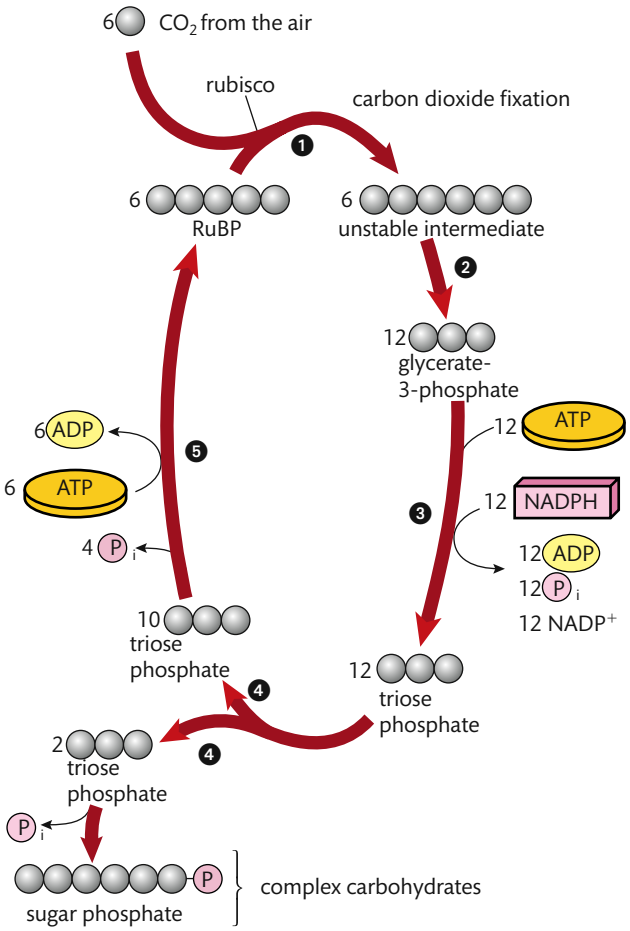


Figure 8.23 The Calvin cycle. The numbered steps are described in the text.

Refer to Figure 8.23 as you read about the steps of the Calvin cycle.

1. Ribulose bisphosphate (RuBP), a 5-carbon compound, binds to an incoming carbon dioxide molecule in a process called carbon fixation. This fixation is catalysed by an enzyme called RuBP carboxylase (rubisco). The result is an unstable 6-carbon compound.
2. The unstable 6-carbon compound breaks down into two 3-carbon compounds called glycerate 3-phosphate (GP).
3. The 3-carbon molecules of GP are acted upon by ATP and NADPH from the light-dependent reaction to form two other 3-carbon molecules called triose phosphate (TP). This is a reduction reaction.
4. The molecules of TP may then go in either of two directions. Some leave the cycle to become sugar phosphates that may become more complex carbohydrates. Most, however, continue in the cycle to reproduce the originating compound of the cycle, RuBP.
5. In order to regain RuBP molecules from TP, the cycle uses ATP.

In Figure 8.23, spheres are used to represent the carbon atoms so that they can be tracked through the cycle. The coefficients (numbers) in front of each compound involved show what it takes to produce one molecule of a 6-carbon sugar. It is clear that for every 12 TP molecules, the cycle produces one 6-carbon sugar and six molecules of the 5-carbon compound RuBP. All the carbons are accounted for, and the law of conservation of mass is demonstrated. Also, it is important to note that 18 ATPs

and 12 NADPH are necessary to produce six RuBP molecules and one molecule of a 6-carbon sugar.

TP is the pivotal compound in the Calvin cycle. It may be used to produce simple sugars such as glucose, disaccharides such as sucrose, or polysaccharides such as cellulose or starch. However, most of it is used to regain the starting compound of the Calvin cycle, RuBP.

NATURE OF SCIENCE

As mentioned earlier, Calvin and his team worked out the details of carbon fixation. In order to do this, Calvin used improvements in apparatus design and recent developments in radioactive tracers and autoradiography. Calvin devised the 'lollipop' apparatus. This is a thin, almost bulb-shaped, glass vessel with a supporting stem. The vessel was designed to mimic the shape of a leaf: thin and broad. He then carried out the following procedures.

- *Chlorella* (a type of green algae) was placed inside the lollipop.
- The algae cells were then exposed to ^{14}C (radioactive carbon) and light.
- Samples of the *Chlorella* were then released from the apparatus at short time intervals.
- Each removed sample was immediately placed into a boiling methanol solution to denature the enzymes and stop the photosynthetic process.
- The compounds within the algae were then separated. Two-way paper chromatography was used for this separation. This process used one solvent to separate the first set of components. Then the paper was turned and placed in a different solvent to obtain a further separation of components.
- The final radioactive products were identified using autoradiography.

Because Calvin carried out this procedure with algae released at different time intervals, he obtained different products at different times. This allowed him to sequence the steps of the overall process and to elucidate the pathways of carbon fixation (the Calvin cycle).

TOK

A team led by Melvin Calvin in the late 1940s and early 1950s worked on experiments to find the early products of photosynthesis. The final products were already well known. His research involved the creation of the now famous 'lollipop' apparatus. This specially designed apparatus was actually a flattened flask that was used to house algal cells carrying out photosynthesis. By using radioactive tracer experiments with this apparatus, Calvin was successful in his studies. Discuss the role of creativity in scientific investigations as well as in art.

Summary of photosynthesis

In summary, the process of photosynthesis includes the light-dependent and the light-independent reactions. The products of the light-dependent reaction are ATP and NADPH, which are needed to allow the light-independent reaction to proceed. Thus it is clear that light is needed for the light-independent reaction to occur, but not directly. A summary of the two reactions is shown in Figure 8.24.

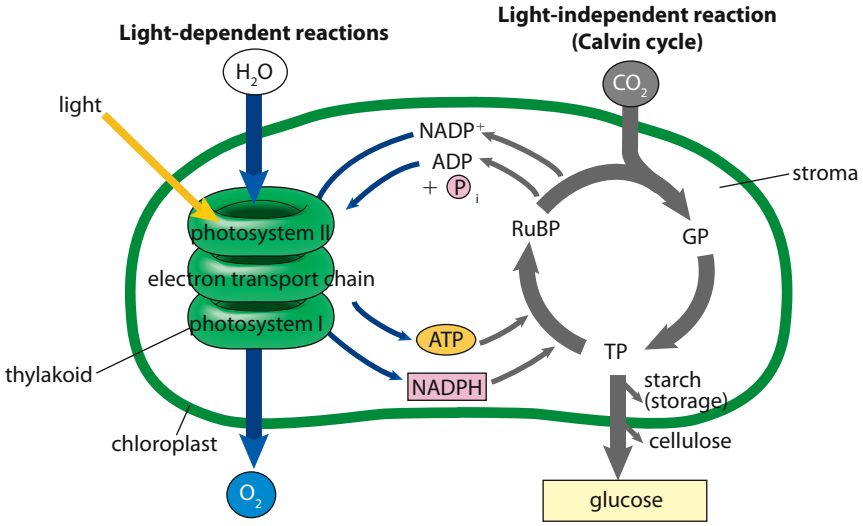


Figure 8.24 A summary of the complete process of photosynthesis.

To learn more about photosynthesis, go to the hotlinks site, search for the title or ISBN, and click on Chapter 8: Section 8.3

Note that $NADP^+$ and ATP move back and forth in the chloroplast from the thylakoids to the stroma in their reduced and oxidized forms. A final summary of the two reactions is shown in Table 8.7.

Table 8.7 Photosynthesis

Light-dependent reaction	Light-independent reaction
Occurs in the thylakoids	Occurs in the stroma
Uses light energy to form ATP and NADPH	Uses ATP and NADPH to form triose phosphate
Splits water in photolysis to provide replacement electrons and H^+, and to release oxygen to the atmosphere	Returns ADP, inorganic phosphate, and NADP to the light-dependent reaction
Includes two electron transport chains and Photosystems I and II	Involves the Calvin cycle

The chloroplast and photosynthesis

From the explanation of photosynthesis, it is clear how important the chloroplast is to the overall process. The structure of the chloroplast allows the light-dependent and light-independent reactions to proceed efficiently. In biology, the relationship of structure to function is a universal theme. The chloroplast and photosynthesis are no exception to this, as shown in Table 8.8.

Table 8.8 The structure and function of a chloroplast

Chloroplast structure	Function allowed
Extensive membrane surface area of the thylakoids	Allows greater absorption of light by photosystems
Small space (lumen) within the thylakoids	Allows for faster accumulation of protons to create a concentration gradient
Stroma region similar to the cytosol of the cell	Allows an area for the enzymes necessary for the Calvin cycle to work in
Double membrane on the outside	Isolates the working parts and enzymes of the chloroplast from the surrounding cytosol

CHALLENGE YOURSELF

3 Examine the diagram of a typical chloroplast (Figure 8.25). Answer the questions below the diagram with the appropriate letter.

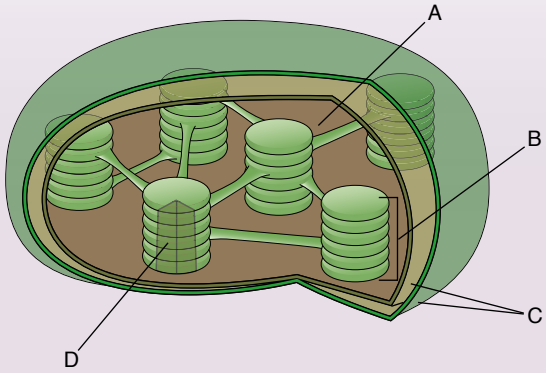


Figure 8.25

(a) Which letter represents the stroma where all the enzymes necessary for the light-independent reaction occur?
(b) Which letter represents the double membrane that controls the entry and exit of materials for the chloroplast?
(c) What is the letter of the thylakoid that contains the photosystems?
(d) Which letter represents a granum, which is where the light-dependent reaction occurs?
(e) Which two letters represent the areas of the chloroplasts that cause the green colour of chloroplasts? Why do these areas create this colour?
(f) The chloroplasts within some plant cells can often be seen moving in a cyclical pattern near the periphery of the cell. This is called cyclosis or cytoplasmic streaming. What might be the value of such movement to the process of photosynthesis?

Scientists in laboratories located around the world are presently working on the development of an artificial leaf. This laboratory-developed leaf would be capable of carrying out photosynthesis. The efforts of these research facilities may one day greatly increase the availability of food resources for the world's hungry human populations.

To become more confident in your understanding of the chloroplast, obtain some electron micrographs of chloroplasts from several different plants. On these micrographs, annotate names of structures and their functions.

Once you understand the details of photosynthesis, return to Section 2.8. Look again at the section about limiting factors of photosynthesis. You should now be able to explain more fully how temperature, light intensity, and carbon dioxide concentration may limit the rate of photosynthesis.

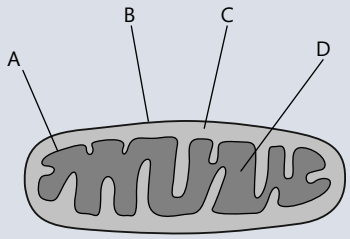
Exercises

9 Why do plants need both mitochondria and chloroplasts?
10 You have a leaf from each of two very different plants. One leaf has more pigments than the other. Which leaf would have the greater photosynthetic rate, assuming all affecting factors are equal? Why?
11 Explain the final products of the two photosystems involved in the light-dependent reaction of photosynthesis.
12 Many scientists state that the enzyme RuBP carboxylase (rubisco) is the most ubiquitous protein on Earth. Why is there a very good chance that this is true?
13 How are the products of the light-dependent reaction important to the light-independent reaction?

The first international conference dedicated to the creation of an artificial leaf was held in 2011. This conference addressed the goals of the Global Artificial Photosynthesis (GAP) project. Research centres are active in this area of study throughout the world, and the objective of this conference was to allow the researchers to share their discoveries. Energy capture, energy conversion and storage, and carbon fixation using modified and synthetic biological processes, were all addressed. From the GAP project, it is hoped that enhanced crop production, reduced atmospheric CO_2 levels, and increased availability of fuels for heating and cooking may be realized.

Practice questions

1 Where is carbon dioxide produced in the mitochondrion?



(*Total 1 mark*)

2 In the mitochondrial electron transport chain, what is the last electron acceptor?

A CO_2
B H_2O
C O_2
D NAD

(*Total 1 mark*)

3 Which of the following statements is **true** about enzymes?

A They are used up in the reactions they catalyse.
B Allosteric inhibitors bind to the active site.
C They lower the energy of activation for a reaction.
D They supply the energy of activation for a reaction.

(*Total 1 mark*)

4 What is the role of NADH + H^+ in aerobic cell respiration?

A To transfer hydrogen to the electron transport chain.
B To reduce intermediates in the Krebs cycle.
C To accept electrons from the electron transport chain.
D To combine with oxygen to produce water.

(*Total 1 mark*)

5 What reaction, involving glycerate 3-phosphate, is part of the light-independent reactions of photosynthesis?

A Glycerate 3-phosphate is carboxylated using carbon dioxide.
B Two glycerate 3-phosphates are linked together to form one hexose phosphate.
C Glycerate 3-phosphate is reduced to triose phosphate.
D Five glycerate 3-phosphates are converted to three ribulose 5-phosphates.

(*Total 1 mark*)

6 What is the advantage of having a small volume inside the thylakoids of the chloroplast?

A High proton concentrations are rapidly developed.
B High electron concentrations are rapidly developed.
C Photosynthetic pigments are highly concentrated.
D Enzymes of the Calvin cycle are highly concentrated.

(*Total 1 mark*)

7 During glycolysis a hexose sugar is broken down to two pyruvate molecules. What is the correct sequence of stages?

A Phosphorylation → oxidation → lysis

B Oxidation → phosphorylation → lysis

C Phosphorylation → lysis → oxidation

D Lysis → oxidation → phosphorylation

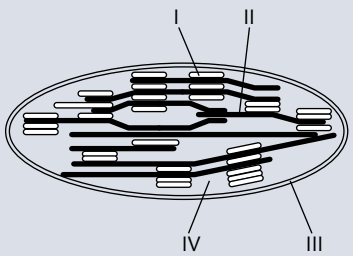
(*Total 1 mark*)

8 Which is correct for the non-competitive inhibition of enzymes?

	Inhibitor resembles substrate	Inhibitor binds to active site
A	yes	yes
B	yes	no
C	no	yes
D	no	no

(*Total 1 mark*)

9 Where are the light-dependent and light-independent reactions taking place in the diagram below?



	Light dependent	Light independent
A	I	IV
B	II	III
C	III	II
D	IV	I

(*Total 1 mark*)

10 What is the link reaction in aerobic respiration?

A Pyruvate is carboxylated, acetyl reacts with coenzyme A, reducing NADH + H^+

B Pyruvate is decarboxylated, acetyl reacts with coenzyme A, forming NADH + H^+

C Pyruvate reacts with coenzyme A, forming NADH + H^+

D Pyruvate is decarboxylated, reacting with coenzyme A, reducing NADH + H^+

(*Total 1 mark*)

09 Plant biology

Essential ideas

9.1	Structure and function are correlated in the xylem of plants.
9.2	Transport in the phloem of plants.
9.3	Plants adapt their growth to environmental conditions.
9.4	Reproduction in flowering plants is influenced by the biotic and abiotic environment.

Flowering plants are so important in our lives. They often make us feel better by just looking at them. They provide us with a variety of foods, and with many other essential materials that we use in our everyday lives.

It is obvious that plants are an extremely important part of life on Earth. They produce oxygen and carbohydrates while absorbing carbon dioxide from our atmosphere. The importance of oxygen and carbohydrates are clear. The removal of carbon dioxide is especially critical because of global warming. There are many, many other contributions of plants as well. They have been a key element in our past and will be equally important in our future. The plant kingdom is vast in its diversity. In this chapter, we will focus on the more highly evolved plants. Included in our study will be transportation within the xylem and phloem, growth, and reproduction.

9.1 Transport in the xylem of plants

Understandings:

- Transpiration is the inevitable consequence of gas exchange in the leaf.
- Plants transport water from the roots to the leaves to replace losses from transpiration.
- The cohesive property of water and the structure of the xylem vessels allow transport under tension.
- The adhesive property of water and evaporation generate tension forces in leaf cell walls.
- Active uptake of mineral ions in the roots causes absorption of water by osmosis.

Applications and skills:

- Application: Adaptations of plants in deserts and in saline soils for water conservation.
- Application: Models of water transport in xylem using simple apparatus including blotting or filter paper, porous pots, and capillary tubing.
- Skill: Drawing the structure of primary xylem vessels in sections of stems based on microscope images.
- Skill: Measurement of transpiration rates using potometers.
- Skill: Design an experiment to test hypotheses about the effect of temperature or humidity on transpiration rates.

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Use models as representations of the real world: mechanisms involved in water transport in the xylem can be investigated using apparatus and materials that show similarities in structure to plant tissues.

Basic leaf structure

When studying water transport in plants, it is essential to understand the leaf's role in this process. Water is lost in the form of a gas from the leaf through openings called stomata (singular stoma). Transpiration is the term given to the loss of water vapour from leaves and other aerial parts of the plant. The water lost from the plant's upper structures must be replaced by water absorption.

As mentioned in Section 2.9, leaves are the main plant organs involved in photosynthesis. They vary greatly in form, but they generally consist of a flattened portion called the blade and a stalk called the petiole that attaches the blade to the stem. A plan diagram, sometimes called a low-power diagram, is shown in Figure 9.1.

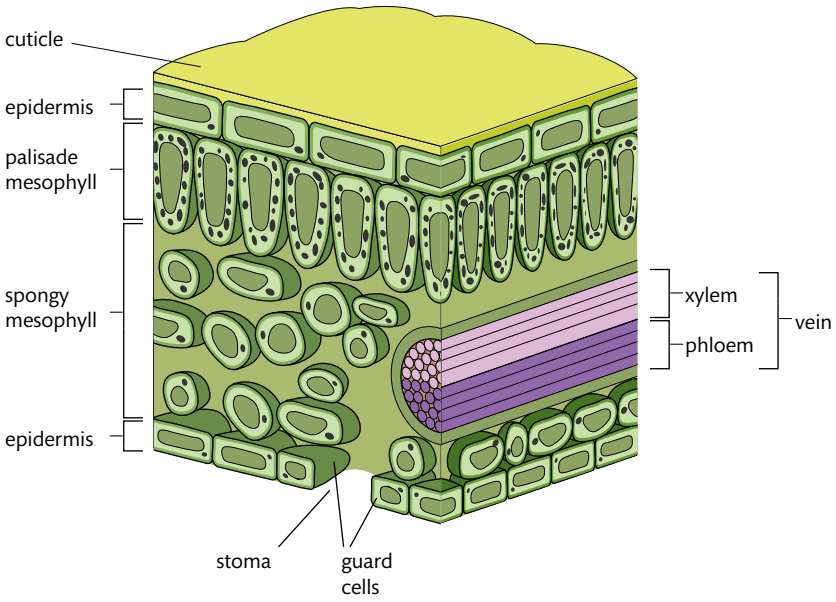


Figure 9.1 Major structures of a generalized leaf.

Many leaves have a layer of wax called the cuticle as their outermost layer. This layer protects the plant against water loss and insect invasion. If a cuticle is not present, the outermost layer is the epidermis that protects the plant. Like stems and roots, the leaves have vascular tissue that includes xylem and phloem. The xylem brings water to the leaves, while the phloem carries the products of photosynthesis to the rest of the plant. The xylem and phloem occur together in veins or vascular bundles. A densely packed region of cylindrical cells occurs in the upper portion of the leaf. This region is called the palisade mesophyll. The cells here contain large numbers of chloroplasts to carry out photosynthesis. The bottom portion of the leaf is composed of the spongy mesophyll. This consists of loosely packed cells with few chloroplasts. There are many air spaces in this area, which provide gas exchange surfaces. Stomata or stomatal pores occur on the bottom surface of leaves, and they allow oxygen and carbon dioxide exchange between the leaf and the surrounding environment. As oxygen and carbon dioxide are exchanged at the stomata, it is an inevitable consequence that water is lost from the plant. Specialized cells called guard cells control the opening and closing of the stomata.

Stomata is the plural form of the word for the small openings in the plant epidermis that allow gas exchange. Stoma is the term for a single opening.

It is important to note the functions of tissues in relation to their position in the leaf.

- The palisade mesophyll is located in the upper portion of the leaf, where light is most available. The cells of this region are chloroplast rich, to allow maximum photosynthesis.
- Veins are distributed throughout the leaf to transport raw materials and products of photosynthesis. The veins occur roughly in the middle of the leaf so that they are near all the leaf cells.
- The spongy mesophyll is located just superior to the stomata, to allow continuous channels for gas exchange.
- The stomatal pores are on the bottom surface of the leaf. This area receives less light, so as a result the temperature here is lower than on the upper surface. The lower temperature minimizes water loss from the pores and the plant, so the lower epidermis usually has a thinner cuticle than the upper epidermis. The positioning of the epidermis is such that the remaining structures of the leaf are protected and supported.

Research has shown that there has been a decrease in stomatal density in many land plants on most of the Earth's continents. This correlates with the increase in atmospheric carbon dioxide levels in the 20th century as a result of humans burning fossil fuels.

This is a lower leaf surface showing epidermal cells, stomata, and guard cells. The guard cells are in pairs and have a curved outer wall. In the centre of the two guard cells that occur together is the stoma (opening), or darker area, through which gas may enter and leave the leaf. Epidermal cells occur between the guard cell–stomata structures.

Plant water and mineral movement

Transpired water has to be replaced by the intake of water at the roots. There is a continuous stream of water from the roots to the upper parts of a plant.

This stream of water through the plant provides minerals to the plant as well as the water necessary to carry out photosynthesis. The water lost by transpiration is important in cooling sun-drenched leaves and stems.

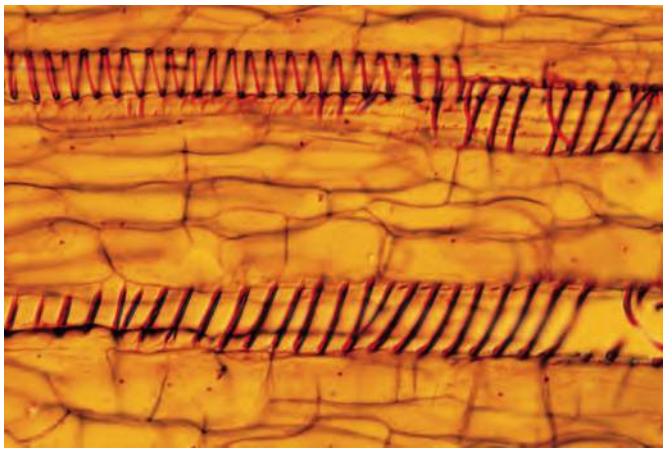
There are many factors involved in the transport of water and minerals in plants. Given the height of some plants, like the sequoias of western America, the transport of water from the roots to the tree top can be a mammoth task. Xylem is involved in supporting the plant as well as being the specialized water-conducting tissue of terrestrial plants.

Xylem is actually a complex tissue composed of many cell types. The two cell types largely involved in water transport are tracheids and vessel elements (see the photo). Tracheids are dead cells that taper at the ends and connect to one another to form a continuous column. Vessel elements (also called vessels) are the most important xylem cells involved in water transport. They are also dead cells, and have thick, lignified secondary walls. These secondary walls are often interrupted by areas of primary wall. These primary wall areas also include pits or pores that allow water to move laterally. The vessel elements are attached end to end to form continuous columns, like the tracheids. The ends of the vessel elements have perforations in them, allowing water to move freely up the plant.

In a typical plant more than 90% of the water taken in by the roots is lost by transpiration. A mountain range in the USA called the Great Smokey Mountains got its name because of the continual haze above it created by transpiration from the abundant trees present.

Sequoiadendron giganteum, the redwood sequoia, is the tallest tree in the world. Individual trees can grow to more than 118 metres (390 feet) and have a trunk diameter of more than 10.5 metres (35 feet). These trees require nearly 1.4 m (55 inches) of rainfall a year to reach their great size.

A light micrograph of a section through pumpkin tissue showing the lignified walls of the vessel elements.



Lignin is a complex organic compound that greatly strengthens the cell walls of vascular plants. It also waterproofs plant parts and adds protection against pathogens. The primary cell wall of plants is made up of cellulose. In some plants, as cells age they form a secondary cell wall composed of materials other than cellulose. One of the materials in this later-forming wall is lignin.

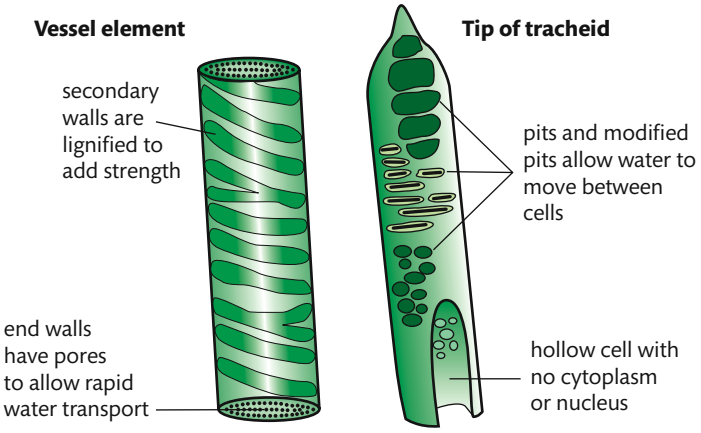


Figure 9.2 Vessel elements and tracheids. Water passes from one tracheid to another through thin regions called pits. In vessel elements, water passes through pits in the primary wall areas and through the end walls. Observations involving tracheids and vessels have provided evidence that vessel elements evolved after tracheids.

Ancient flowering plants only had tracheids, while most modern flowering plants only have vessel elements. Vessel elements appear to be more efficient in the transport of water.

The most widely accepted explanation for the movement of water and minerals upwards in plants has several names. Some biologists call it the transpiration–cohesion–tension mechanism, while others call it simply the cohesion–tension theory.

Using prepared microscope slides or photomicrographs of stem or root cross-sections of various plant types, draw and label the primary xylem vessels. Careful observation should reveal the thick walls with pits or pores that allow lateral and vertical movement of water.

A 'living' model to observe water transport in plants

Safety alerts: Wear eye protection and aprons during this procedure. It is recommended to use protective gloves when handling the Congo red or red ink. Follow all directions from your teacher in the disposal of all chemicals used. Wash your hands thoroughly after completing this lab.

The plant species *Impatiens wallerana* has translucent stems because of the presence of large, thin-walled cells. This species can be grown from seed or bought from a local florist. Follow these general procedures.

- Carefully remove the plant and its root system from the soil.
- Gently wash the roots and place them in a solution of Congo red (red ink could also be used).
- Stabilize the plant in an upright position, being careful not to put too much pressure on any part of the stem.
- Observe the stem for movement of the coloured solution.

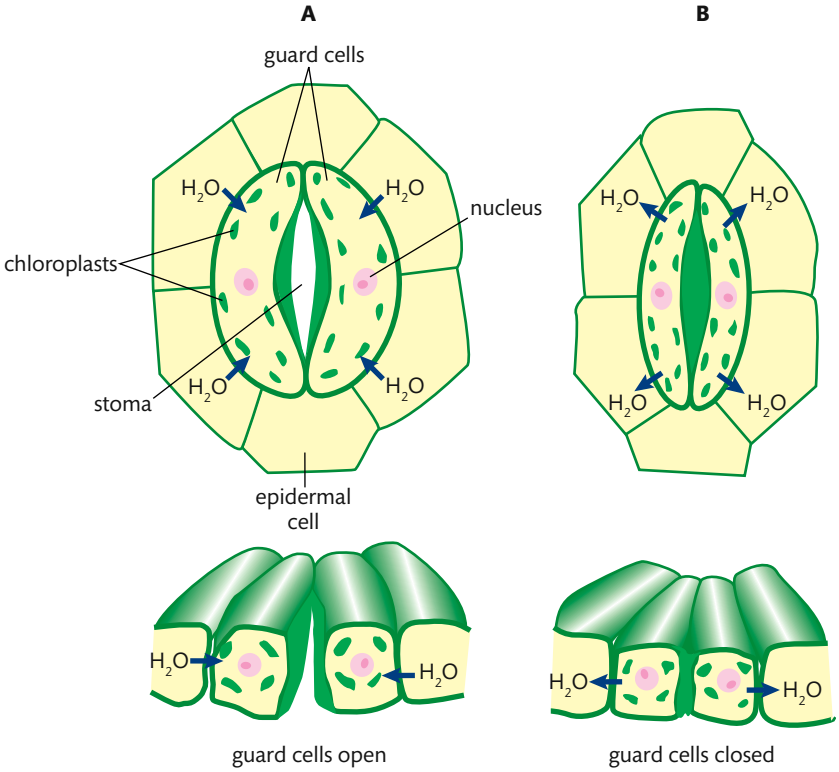
Now answer the following questions.

1 Explain several factors that may play a role in the movement observed in this 'living' model.

2 Suggest whether the xylem of this particular plant has lignin present in a high amount. Explain how you arrived at this conclusion.

Stomata and guard cells

Stomata can only be closed on a short-term basis. This is because carbon dioxide must enter the mesophyll region of the leaf so that photosynthesis can occur. The stomata open and close because of changes in the turgor pressure of the guard cells that surround them. These guard cells are cylindrical and their cell wall thickness is uneven. As you can see in Figure 9.3, the thickened area of the guard cell wall is oriented towards the stoma. Thus when the cells take in water and swell, they bulge more to the outside. This opens the stoma. When the guard cells lose water, they sag towards each other and close the stoma.



The gain and loss of water in the guard cells is largely because of the transport of potassium ions. Light from the blue part of the light spectrum triggers the activity of adenosine triphosphate (ATP)-powered proton pumps in the plasma membrane of guard cells. This triggers the active transport of potassium into the cell. The higher solute concentration within the guard cells causes inward water movement by osmosis.

When potassium ions passively leave the cells, water also leaves. The plant hormone abscisic acid causes potassium ions to diffuse rapidly out of the guard cells. The result is stomatal closure. This hormone is produced in the roots during times of water deficiency, for example during a drought.

Other factors, such as carbon dioxide levels and even circadian rhythms (the basic 24-hour biological clock) within plants, affect stomata opening and closing.

The cohesion–tension theory of plant fluid movement

Now that the structures of the plant involved in fluid movement have been described, we can explain the cohesion–tension theory of plant fluid movement. Table 9.1 and Figure 9.4 show how it works.

NATURE OF SCIENCE

Models are often used to represent the movement of water in plants. Simple apparatus, such as blotting or filter paper, porous pots and capillary tubing, can be used. Often models use organisms in a novel way to represent a particular process. An example of such a model is provided in the *Impatiens wallerana* lab.

Turgor refers to the pressure in a cell that liquid exerts on the membrane and/or cell wall.

Figure 9.3 A: When solution pressure is high inside the guard cells, they bow outwards and open the stoma. B: When solution pressure is low, the guard cells become limp and the stoma closes.

Table 9.1 The cohesion–tension theory of plant fluid movement

Process	Explanation
Water moves down concentration gradients	The spaces within a leaf have a high concentration of water vapour. Water moves from this location to the atmosphere, which has a lower water concentration
Water lost by transpiration is replaced by water from the vessels	Replacing water from the vessels maintains a high water vapour concentration in the air spaces of the leaf
The vessel water column is maintained by cohesion and adhesion	Cohesion involves the hydrogen bonds that form between water molecules. Adhesion involves the hydrogen bonds that form between water molecules and the sides of the vessels; adhesion counteracts gravity
Tension occurs in the columns of water in the xylem	This is because of the loss of water in the leaves and the replacement of that lost water by xylem water. The water columns remain continuous because of cohesion and adhesion
Water is pulled from the root cortex into xylem cells	Cohesion and adhesion maintain the columns under the tension created by transpiration
Water is pulled from the soil into the roots	This happens because of the tension created by transpiration and the maintenance of a continuous column of water

CHALLENGE YOURSELF

1 The properties of water are very important in allowing fluid movement from the roots to the top of a plant. Why does water form hydrogen bonds between its molecules and the sides of the xylem vessels?

2 Early research indicated that fluid could move in plants even when roots were removed. Leaves, however, are essential for fluid transport. According to the cohesion–tension theory, why are leaves essential to fluid transport in plants?

3 It has been found that some trees and shrubs are able to live in seawater. An example of this is the coastal mangrove, which lives in markedly hypertonic seawater. (Hypertonic and hypotonic were discussed in Section 1.4.) According to the cohesion–tension theory, how might these remarkable plants be able to survive in this very difficult environment?

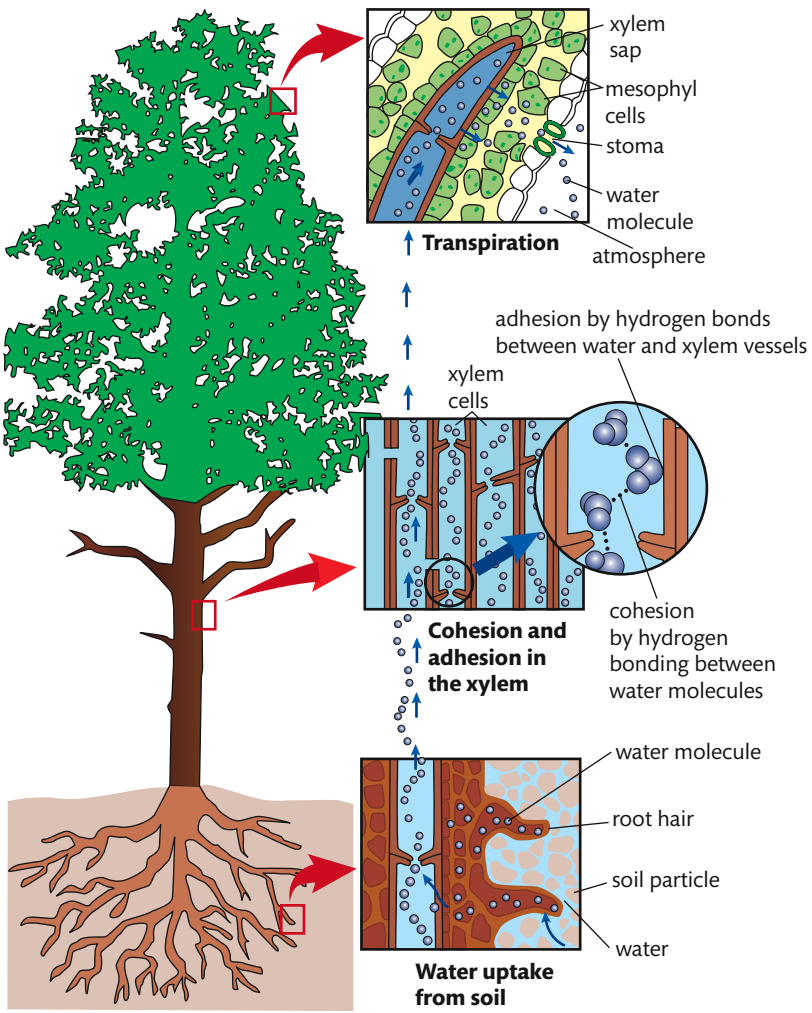


Figure 9.4 The mechanism for upward movement of water in land plants.

Roots and fluid movement in plants

The main function of roots is to provide mineral ion and water uptake for the plant. Roots are efficient at this because of an extensive branching pattern, and because of some specialized epidermal structures called root hairs.

Root hairs increase the surface area over which water and mineral ions can be absorbed by a factor of nearly three. The root cap is very important in protecting the apical meristem during primary growth of the root through the soil. The three root zones indicate regions of cell development.

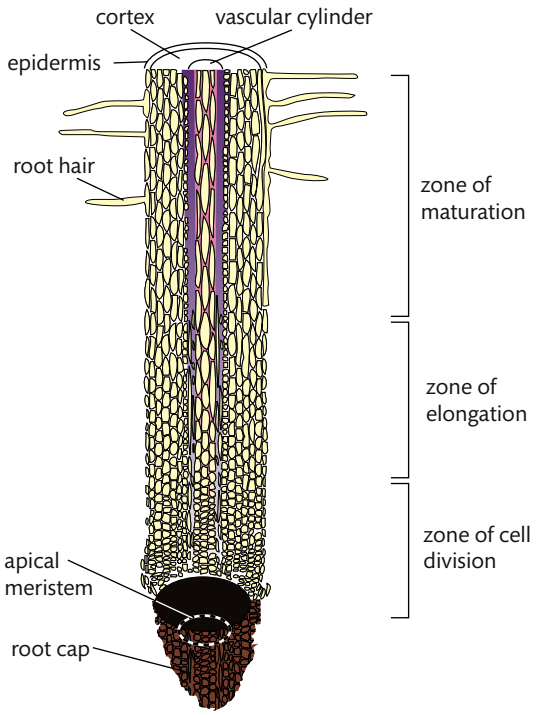


Figure 9.5 This is a root tip. Note the root hairs developing in the zone of maturation.

- The zone of cell division is where new undifferentiated cells are forming, corresponding with the M phase of the cell cycle (see Section 1.6).
- The zone of elongation is where cells are enlarging in size, corresponding with the G_1 phase of the cell cycle.
- The zone of maturation is where cells become a functional part of the plant.

Water moves into the root hairs from the soil because the root hairs have a higher solute concentration and a lower water concentration than the surrounding soil. Therefore, the water moves through the plasma membranes into the root hair cells.

Most of the water entering a plant comes in through the root hairs by osmosis. Once in the root, water moves to the vascular cylinder, which contains the xylem and phloem.

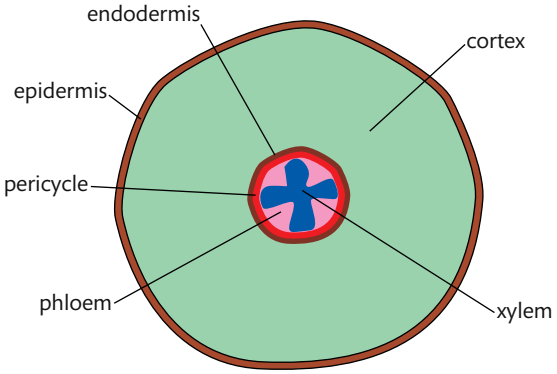


Figure 9.6 This is a cross-section through a root.

An adult rye plant was found to have a root system with 14 million branches totalling 630 kilometres (380 miles) in length.

As mentioned on page 10, meristematic cells are undifferentiated cells that can divide rapidly, allowing growth in plants. There are two major types of meristematic tissue, apical and lateral. Apical meristematic tissue occurs in root tips and in other areas that allow lengthening of plant parts. Lateral meristematic tissue occurs in stem tissue and allows growth in width.

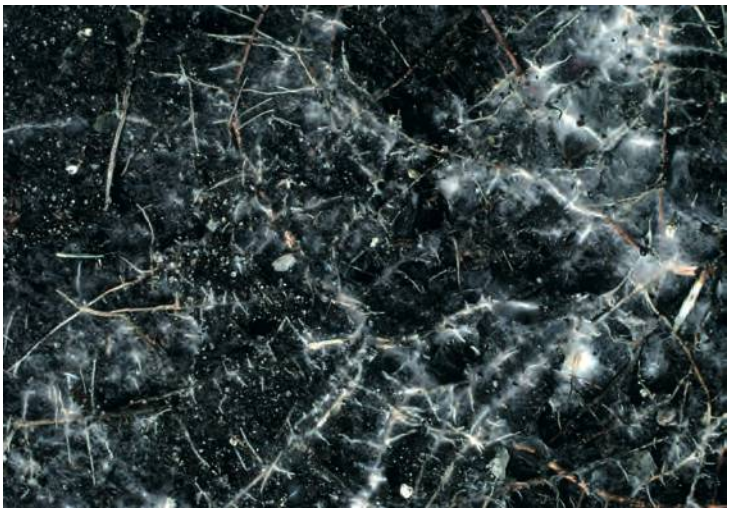
Mineral ions

It is essential that mineral ions move into the root as well as water. There are three major processes that allow mineral ions to pass from the soil to the root:

- diffusion of mineral ions and mass flow of water in the soil that carries these ions
- the action of fungal hyphae
- active transport.

When there is a higher concentration of a mineral dissolved in water outside the root than inside, the mineral ions may move passively, without cell ATP expenditure, into the root cells. This is an example of diffusion. Also, some of the minerals dissolved in the water will move into the roots as water moves into the outer root cells via osmosis. This passive flow of water and the minerals dissolved in it is referred to as bulk flow or mass flow.

The help provided by fungal hyphae to plants is unique: there is a symbiotic relationship between some roots and fungi. Large numbers of fungal filaments called hyphae form a cover over the surface of young roots. This creates an even larger surface area for water and mineral ion absorption. The result of this mutualistic relationship is referred to as a mycorrhiza; an example is shown in the photo below.



Mycorrhizal fungi growing in association with roots.

Often there is a higher concentration of various mineral ions inside the plant than outside. In this situation, the passive means of transport mentioned so far are not useful. If the plant is to absorb these minerals, active transport is needed. This requires energy.

Another very common reason why a plant's roots may have to expend energy for a particular mineral ion to pass into it, is because the ion cannot cross the lipid bilayer of the membranes. In this instance, the ions must pass through a transport protein in the membrane. These transport proteins are specific for certain ions. They bind to the ion on one side of the membrane and then release it on the other side. This requires energy. Potassium ions move through specialized transport proteins called potassium channels.

The result of the active uptake of mineral ions is a high solute (hypertonic) concentration within the root. Because of this, the amount of water absorbed from the soil by the root through the process of osmosis is increased.

The proton pump may be used to transport mineral ions and solutes such as potassium ions, nitrogen-based ions, and even simple sugars. This pump represents a form of chemiosmosis, as talked about in respiration and photosynthesis (Chapter 8). In this case, however, the process is in reverse. Instead of ATP being formed by the movement of hydrogen ions, ATP is broken down to allow various mineral ions to move into the root cells.

Plant adaptations for water conservation

The transpiration process is affected by a number of environmental factors, as summarized in Table 9.2.

Table 9.2 Environmental effects on transpiration in most plants

Environmental factor	Effect
Light	Speeds up transpiration by warming the leaf and opening stomata
Humidity	Decreasing humidity increases transpiration because of the greater difference in water concentration
Wind	Increases the rate of transpiration because humid air near the stomata is carried away
Temperature	Increasing temperature causes greater transpiration because more water evaporates
Soil water	If the intake of water at the roots does not keep up with transpiration, turgor loss occurs and the stomata close, and the transpiration rate decreases
Carbon dioxide	High carbon dioxide levels in the air around the plant usually cause the guard cells to lose turgor and the stomata to close

Plants that survive in desert and high saline environments have adaptations that allow their survival. These adaptations allow water conservation.

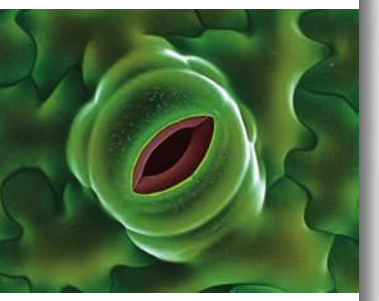
Xerophytes are plants adapted to arid climates. They have an impressive list of adaptations to reduce transpirational water loss.

- Small, thick leaves reduce water loss by decreasing the surface area of the leaves.
- A reduced number of stomata decreases the number of openings through which water loss may occur.
- Stomata are located in crypts or pits on the leaf surface. This causes higher humidity near the stomata.
- A thickened, waxy cuticle reduces water loss by acting as an impenetrable barrier to water.
- Hair-like cells on the leaf surface trap a layer of water vapour, thus maintaining a higher humidity near the stomata.
- Many desert plants shed their leaves and/or become dormant in the driest months.
- Cacti exist on water that the plant stores in fleshy, watery stems. Plants of this type are called succulents. This stored water is obtained in the rainy season.
- Xerophytes can use alternative photosynthetic processes. In Section 8.3 the most common form of photosynthesis was explained in detail. It is known as the C_3 photosynthetic pathway. There are two alternative processes, called CAM photosynthesis and C_4 photosynthesis. CAM plants close stomata during the day and incorporate carbon dioxide during the night. C_4 plants have stomata that open during the day but take in carbon dioxide more rapidly than non-specialized plants.

Halophytes are plants adapted to grow in water with high levels of salinity. Some of these plants are being studied for use as the next generation of biofuel. They are

Some plants have specialized outgrowths of hair-like structures called trichomes which allow retention of water by slowing water loss from the plant. They may also serve to reflect light thus allowing a lower leaf temperature and less water loss. In many plants, these trichomes may even secrete chemicals which protect against pathogens and even herbivores.

Guard cells and open stoma of a tobacco leaf.



a promising source of biofuel because they do not compete with food crops for resources. Again, halophytes have an impressive list of adaptations.

- Many become succulent by storing water, thus diluting the salt concentrations.
- Several species, for example the mangrove, secrete salt through salt glands.
- Some species are able to compartmentalize Na^+ and Cl^- in the vacuoles of their cells, thereby preventing NaCl toxicity.
- Sunken stomata on thickened leaves reduce water loss by creating a higher humidity near the stomata. The thickened leaves often include a more developed cuticle to minimize water loss.
- The surface area of the leaves is reduced.

One final adaptation of both halophytes and xerophytes to reduce water loss is to simply close the stomata using the action of the guard cells.

TOK

If higher temperatures are the result of global warming, what effect might global warming have on plant transpiration rates? How might this affect food availability around the world?

Measurement of transpiration rates using potometers

Safety alerts: Use protective eyewear and aprons. Be very cautious with sharp instruments. Follow all additional teacher safety directives. Wash your hands thoroughly with soap and water before and after handling the plants.

A potometer is a device for investigating transpiration rate (water loss per unit time). An example of an idealized potometer is shown in Figure 9.7.

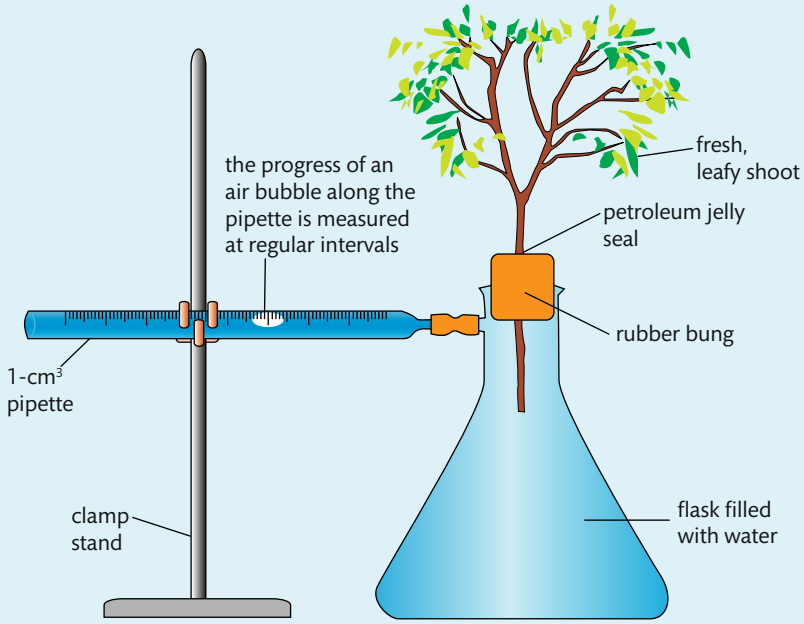


Figure 9.7 An example of a potometer used to measure transpiration rate.

There are many different designs for potometers. The design presented in the book is one of the easiest to set up and get measurements with.

There are several factors to consider when setting up potometers.

- Use shoots from a plant with leaves with thin waxy cuticles. Thick waxy cuticles decrease the chance of meaningful data.
- The shoots must be cut under water. This will prevent air from entering the xylem vessels of the plant which would block water flow. Be certain to completely seal the contact point between the shoot and the apparatus with petroleum jelly. Also, the potometer must be assembled under water, again to prevent air bubbles in the apparatus. This will require a large sink.

- Before meaningful readings may occur, the leaves must be dry. After the apparatus is assembled, a calibration period is necessary which will allow evaporation or loss of water from the leaves. The calibration period may be shortened by carefully drying the leaves with paper toweling.
- If an apparatus is being prepared which will utilize the movement of an air bubble for readings, a calibration period of 15 to 20 minutes should be allowed for the plant and apparatus. Then lift the potometer pipette or glass tube out of the water for a few minutes until a large air bubble is visible. Then lower the pipette or glass tube back into the water. Measurements may then be taken.

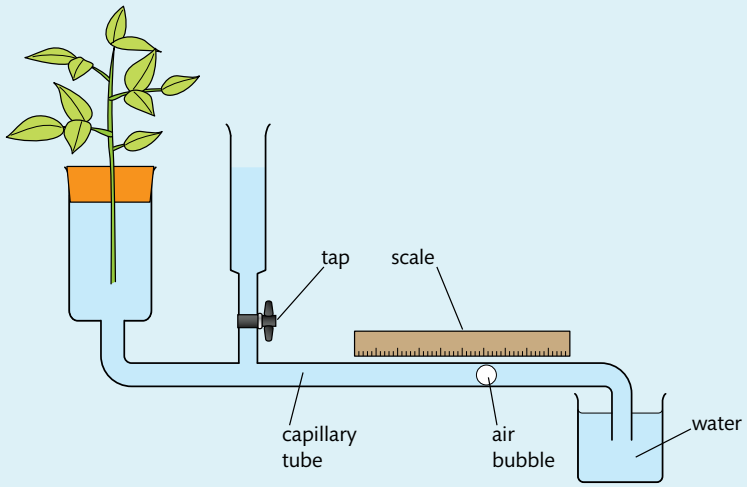


Figure 9.8 This is another model of a potometer which may be used for this practical. This potometer uses an air bubble for readings.

Another type of potometer is a mass potometer. In this design, a complete plant with its whole root system is immersed in a container. A layer of mineral oil may be placed on the surface of the water container to minimize loss of water by evaporation. The container is then placed on a digital balance in order to find the amount of water lost by transpiration. This technique measures only water lost from the plant by transpiration and does not measure water intake by the plant.

Factors which may affect transpiration rate and may be measured using a potometer include light intensity, wind speed, temperature, leaf surface area, and stomatal density on the surface of different types of leaves. If stomatal density is being tested, a method to count the stomata per unit area would be added.

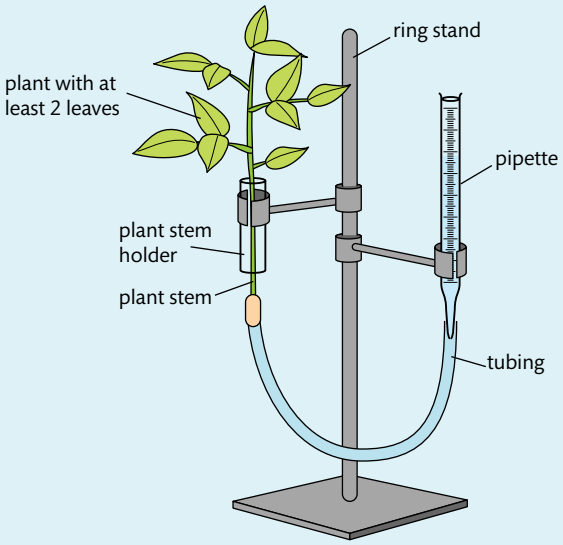


Figure 9.9 Assemble a potometer as shown here.

The following materials are necessary to assemble this potometer:

- clear plastic tubing
- a ring stand with appropriate clamps
- a 0.1-millilitre (ml) or 1.0-ml pipette, depending on the size of the plant stem you will be using and the plastic tubing
- a syringe to pull water into the potometer
- petroleum jelly or small clamps to seal between the cut end of the stem and the tubing.

Once the potometer is assembled, select one factor and determine its effect on the rate of transpiration in the plant species you have chosen. Factors to consider include temperature, humidity, moving air, and high light conditions, to name just a few.

Table 9.3 Sample results

Time	0 min	2 min	4 min	6 min	8 min	10 min	12 min	14 min
Pipette reading (ml)								
Water loss (ml)								
Water loss (ml) per m^2 of leaf surface								

To calculate water loss per square metre of leaf surface, two methods can be used: the leaf trace method using graph paper, or the leaf mass method. Once the leaf surface has been determined in cm^2, convert this value to m^2 using the following formula:

leaf surface area in m^2 = total leaf surface $cm^2/10\,000\ cm^2\ m^{-2}$

This information allows you to fill in the last row of the table.

Once you have your results, complete the following:

3 Draw a graph of your results.

4 Describe the shape of your graph, especially noting the effects of the variable applied for your procedure.

5 Explain in biological terms what your graph shows.

6 Suggest some ways this procedure could be improved.

7 Relate your data to the needs of plants concerning water in their natural environment.

Exercises

1 When moving a plant from one place to another, suggest why is it important to leave some original soil around the roots.

2 What is the usual cause of a plant wilting?

3 Why is transpiration an inevitable consequence of gas exchange in the leaf?

4 How does the active uptake of mineral ions from the soil by plant roots increase the ability of plant roots to absorb water?

To learn more about parts of plants, go to the hotlinks site, search for the title or ISBN and click on Chapter 9.

9.2 Transport in the phloem of plants

Understanding:

- Plants transport organic compounds from sources to sinks.
- Incompressibility of water allows transport along hydrostatic pressure gradients.
- Active transport is used to load organic compounds into phloem sieve tubes at the source.
- High concentrations of solutes in the phloem at the source lead to water uptake by osmosis.
- Raised hydrostatic pressure causes the contents of the phloem to flow towards sinks.

Applications and skills:

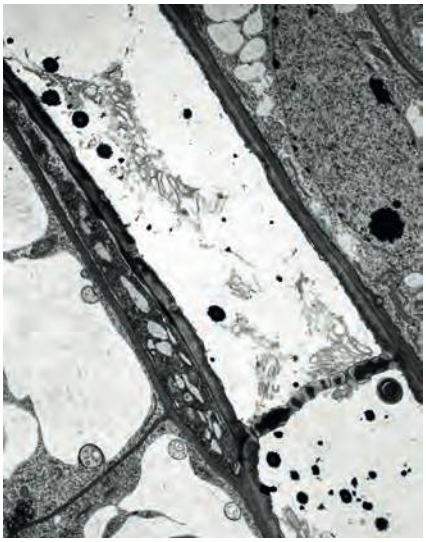
- Application: Structure–function relationships of phloem sieve tubes.
- Skill: Identification of xylem and phloem in microscope images of stem and root.
- Skill: Analysis of data from experiments measuring phloem transport rates using aphid stylets and radioactively labelled carbon dioxide.

NATURE OF SCIENCE

Developments in scientific research follow improvements in apparatus: experimental methods for measuring phloem transport rates using aphid stylets and radioactively labelled carbon dioxide were only possible when radioisotopes became available.

The movement of organic molecules in plants

Organic molecules move in plants via the phloem. Unlike the xylem, phloem is made up of living cells (see Figure 9.10).



This is a transmission electron micrograph (TEM) of a phloem sieve tube and accompanying companion cell.

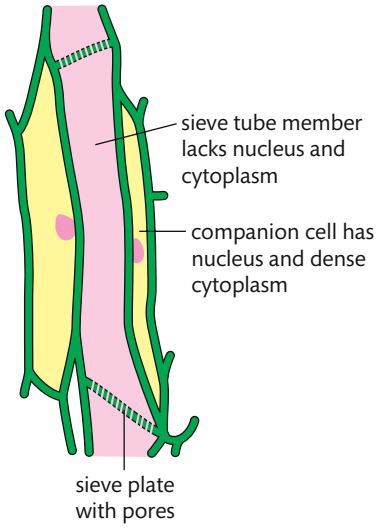


Figure 9.10 The structure of phloem, including the sieve tube member and accompanying companion cell.

Phloem is mostly made up of sieve tube members and their companion cells. Sieve tube members are connected to one another by sieve plates to form sieve tubes. Sieve tubes are referred to by many as sieve elements. The sieve plates have pores that allow the movement of water and dissolved organic molecules throughout the plant. Companion cells are actually connected to their sieve tube members by plasmodesmata.

Whereas xylem cells conduct water and minerals only upwards from the roots, phloem cells transport their contents in various directions. However, the direction of

movement is based on a single principle: the movement is from a source to a sink. A source is a plant organ that is a net producer of sugar, either by photosynthesis or by the hydrolysis of starch. Leaves are the primary sugar sources. A sink is a plant organ that uses or stores sugar. Roots, buds, stems, seeds, and fruits are all sugar sinks. It is possible for some structures to be both a source and a sink. For example, a tuber or bulb may be storing sugar or breaking down starch to provide sugar, depending on the season: tubers and bulbs act as sinks in the summer and as sources in the early spring.

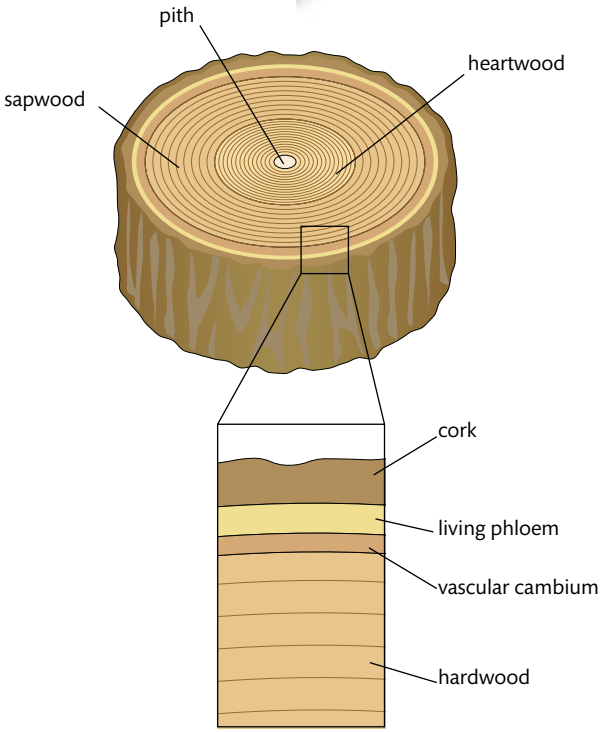


Figure 9.11 When girdling is carried out, the bark and the living phloem just below it is removed.

As early as the 1600s, it was observed that trees slowly die when a ring of bark around the trunk is removed. After the removal of the bark, called girdling, a swelling occurs just above the location of the girdle. First the bark below the girdle, and then the entire tree, dies (see Figure 9.11).

When the living phloem is removed by girdling, the flow of food from the leaves to the roots stops. The swelling is from the trapped sugar solution from the leaves. This is the cause of death of the tree. This practice has often been used to kill unwanted trees.

The movement of organic molecules in plants is called translocation. The organic molecules are dissolved in water and the solution is referred to as phloem sap. The organic molecules of the phloem sap include:

- sugars (sucrose is the most common, and sugars account for most of the phloem)
- amino acids
- plant hormones
- small RNA molecules (this is a recent finding and may explain how cells that are far apart in a plant can communicate).

Plant exudate is any fluid that moves out of the normal transport system of plants. It may include resin, saps, gums, and latex. Nectar is considered by some to be an example of a plant exudate. In some cases these exudates harden over time.

NATURE OF SCIENCE

Two relatively new techniques have allowed major developments in the analysis of phloem sap. The techniques involve the use of aphid stylets and radioactively labelled carbon dioxide. The original methods used to examine phloem contents involved studying the exudates from severed sieve tubes. However, this fluid seemed to contain many contaminants. One technique being used now involves the use of aphids. Aphids feed by inserting structures from their mouth called stylets into a sieve tube. The pressure in the sieve tube forces the contents into the stylet and insect's gut. If a feeding insect is anaesthetized, the body can be cut from the stylet with a laser. The exudate, mostly free of contaminants, can then be analysed. Another relatively new method for studying phloem sap is to use a radioactive form of carbon so that the location of carbon dioxide-fixing reactions of photosynthesis can be determined. A process called autoradiography can then be used to track the flow of the carbohydrate, usually sucrose, through the plant.

The pressure-flow hypothesis

The phloem sap can move as fast as 1 m per hour. Radioactive tracers, mentioned in the Nature of Science, can be used to study this movement, and show that more than just diffusion and osmosis are involved. The best explanation at present for the movement of phloem sap is the pressure-flow hypothesis. It includes the following processes.

- Loading of sugar into the sieve tube at the source. This reduces the relative water concentration in the sieve tube members, causing osmosis from the surrounding cells.
- The uptake of water at the source causes a positive pressure, referred to as hydrostatic pressure, in the sieve tube, which results in a flow (bulk flow) of the phloem sap.
- This hydrostatic pressure is diminished by the removal of sugar from the sieve tube at the sink. The sugars are changed at the sink to starch. Starch is insoluble and exerts no osmotic effect.
- Xylem recycles the relatively pure water by carrying it from the sink back to the source.

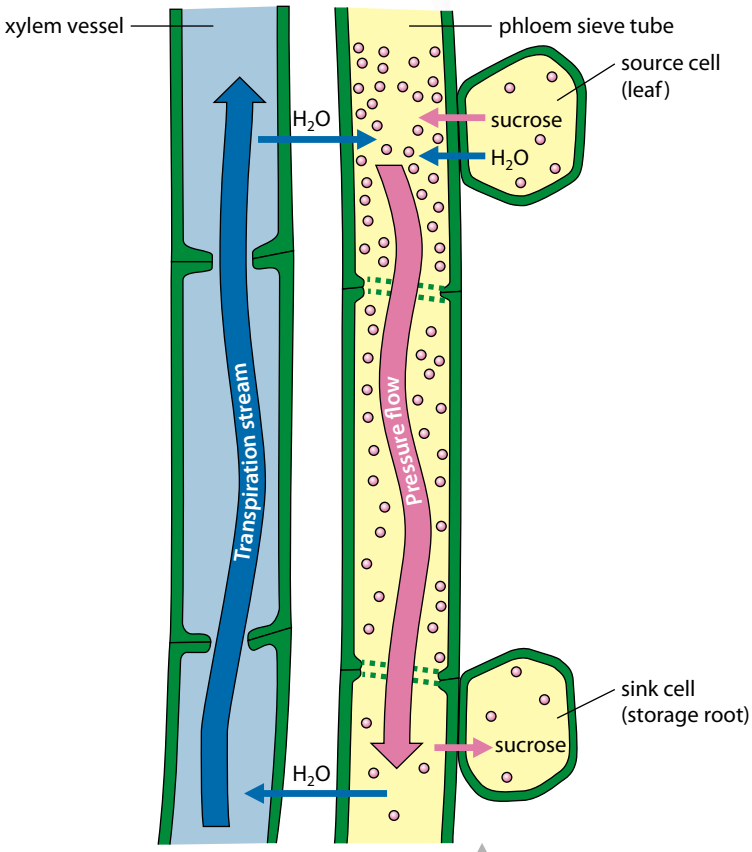


Figure 9.12 The pressure–flow hypothesis.

The loading of sugar into the sieve tube at the source, and the removal of sugar at the sink, is accomplished by active transport. This active transport is a chemiosmotic process involving proton pumps and specialized membrane proteins called cotransport proteins that can allow both passive and active transport. The companion cells of the phloem are involved with the active transport process. Only the loading and removal of sugar from the sieve tube members requires energy: the actual transport in the tube is a passive process. It is passive because it involves transport along hydrostatic pressure gradients. Hydrostatic pressure is produced by compression of a liquid in a confined space or by the addition of solute particles to a liquid in a confined area. As water is relatively incompressible, adding solutes to a limited space filled with water increases pressure. Two areas with different hydrostatic pressure produce a hydrostatic pressure gradient. Water with its dissolved solutes will move from the higher pressure area to the lower pressure area. It is essential that you remember that phloem tissue occurs in all parts of the plant, not just the stem. The leaves are usually source regions because they are the major organs of photosynthesis.

The pressure that occurs within the phloem, as well as the composition of phloem sap, has been demonstrated using the stylets of aphids in the method described above.

From studies using aphid stylets, it is obvious that phloem sieve tubes are hollow, allowing phloem sap to move within the plant. These conducting tubes are actually living cells without a nucleus. Sieve tube elements that make up the sieve tubes have sieve plates with pores at their ends, to allow phloem sap transport to take place from one element to the next. Studies have also indicated the need for ATP production so that sugars can be actively loaded into the phloem source. This ATP is provided by the companion cells, which occur adjacent to the sieve tubes. The companion cells have nuclei and carry out most of the common cell functions. Besides providing ATP, these companion cells are responsible for keeping the sieve tube members alive.

CHALLENGE YOURSELF

Figure 9.13 is a model representing the pressure-flow hypothesis of phloem transport. Study the diagram and answer the accompanying questions.

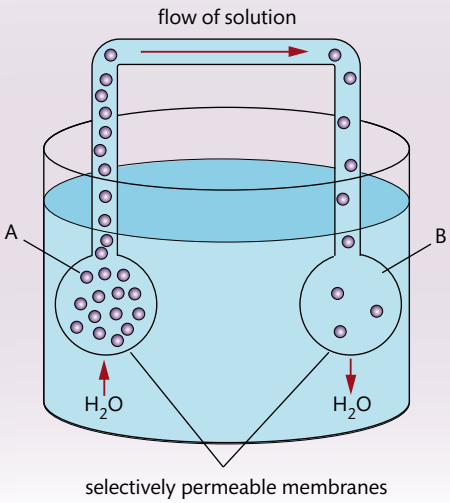


Figure 9.13

4 Which letter represents the source, and which letter represents the sink?

5 Usually, what part of the plant is represented by A?

6 At which letter is hydrostatic pressure the greatest?

7 There are more dots in A than in B. What do these dots probably represent?

8 Explain why the flow of solution from A to B is a passive process.

9 Hydrostatic pressure builds in A because water is incompressible. What would happen at A if water was compressible?

Identification of xylem and phloem in microscopic images of roots and stems

One of the skills important for success in this chapter is the ability to identify xylem and phloem tissue using a microscope. You should be able to differentiate between xylem and phloem in both stem and root cross-sections. If prepared slides of cross-sections of stems and roots are available to you, use them to practise differentiating xylem and phloem. If you do not have access to prepared slides, you can use images from the internet.

Use Figures 9.14 and 9.15 to help locate xylem and phloem tissue in root and plant cross-sections.

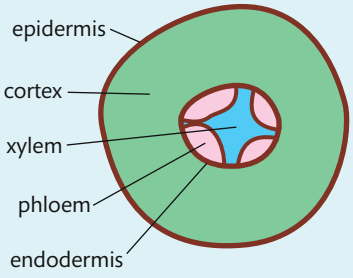


Figure 9.14 A typical plant root and its parts. Diagrams like this one of a root are called plan diagrams, sometimes low-power diagrams. The main purpose of such diagrams is to show the position of different tissues. Individual cells are not shown.

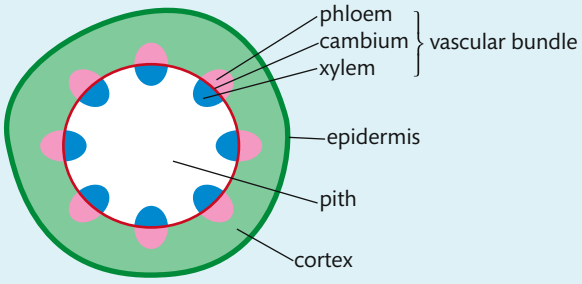


Figure 9.15 This shows the distribution of tissues in a stem of a dicotyledonous plant. Dicotyledonous and monocotyledonous plants and their characteristics will be discussed in Section 9.4

After looking at some prepared slides or internet images, answer the following questions.

8 Does xylem or phloem tissue have hollow tubes with the greater diameter?

9 Are living cells present with the open tubes of xylem?

10 Are living cell present with the open tubes of phloem?

11 Which type of tissue is usually closer to the exterior of the root or stem?

Worked example

Look at these diagrams of tissue from a section of phloem and xylem.

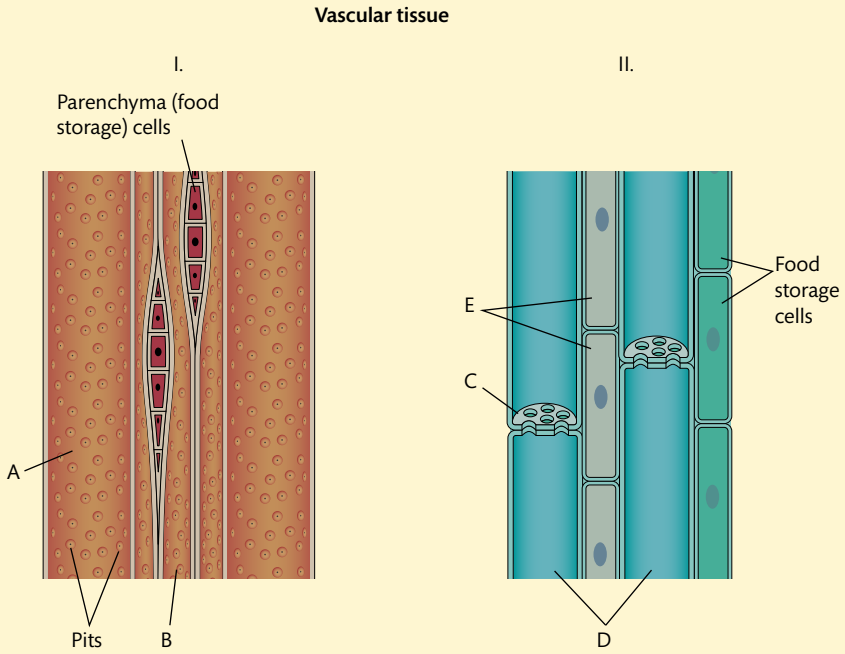


Figure 9.16
http://www.deftstudios.com/bioweb/images/bimg25sm.jpg

1. What type of vascular tissue is I? What type of vascular tissue is II?
2. What is the structure labelled A called?
3. What is the structure labelled D called?
4. What is B?
5. Do sieve plates, labelled C, occur in xylem vessels?
6. What are the structures labelled E? Why are they important to the major function carried out by phloem?

Solutions

1. I = xylem, II = phloem.
2. Vessel element.
3. Sieve tube member.
4. Tracheid.
5. No.
6. Companion cells. Produce the ATP necessary to develop the positive hydrostatic pressure at the source. They also allow the survival of the sieve tube member they are adjacent to.

Exercises

5. Explain when a seed would be a sink and when it would be a source.
6. Why is it necessary for veins to be relatively close together in leaves?

To learn more about transport and vascular tissue in plants, the pressure–flow hypothesis, and to differentiate between xylem and phloem in plant roots and stems, go to the hotlinks site, search for the title or ISBN, and click on Chapter 9.2

NATURE OF SCIENCE

Developments in scientific research follow improvements in analysis and deduction: improvements in analytical techniques allowing the detection of trace amounts of substances have led to advances in the understanding of plant hormones and their effect on gene expression.

9.3 Growth in plants

Understandings:

- Undifferentiated cells in the meristems of plants allow indeterminate growth.
- Mitosis and cell division in the shoot apex provide cells needed for extension of the stem and development of leaves.
- Plant hormones control growth in the shoot apex.
- Plant shoots respond to the environment by tropisms.
- Auxin efflux pumps can set up concentration gradients of auxin in plant tissue.
- Auxin influences cell growth rates by changing the pattern of gene expression.

Applications and skills:

- Application: Micropropagation of plants using tissue from the shoot apex, nutrient agar gels, and growth hormones.
- Application: Use of micropropagation for rapid bulking up of new varieties, production of virus-free strains of existing varieties, and propagation of orchids and other rare species.

Guidance

- *Auxin is the only named hormone that is expected.*

Plant tissues and meristems

Most plants have three basic types of tissues:

- dermal tissue, which is an outer covering that protects against physical agents and pathogenic organisms, prevents water loss, and may have specialized structures for various purposes
- ground tissue, which consists mostly of thin-walled cells that function in storage, photosynthesis, support, and secretion
- vascular tissue, made up of xylem and phloem that carry out long-distance conduction of water, minerals, and nutrients within the plant, and provide support.

These three tissue types are all derived from meristematic tissue. Meristematic tissue is composed of aggregates of small cells that have the same function as stem cells in animals. When these cells divide, one cell remains meristematic while the other is free to differentiate and become a part of the plant body. By doing this, the population of meristematic cells is continually renewed. The cells that remain meristematic are referred to as initials, while the cells that begin differentiation are called derivatives.

Plants are different from most animals in that they show growth throughout their life. This continual pattern of growth is referred to as indeterminate. Animals (and even some plant organs such as leaves) exhibit determinate growth. Determinate growth means that growth ceases after a certain size has been reached. Although plants continue to grow throughout their lives, they do die. Death occurs based on the plant's life cycle. Some plants are annuals and complete their life cycle in one year and then die. Other plants are biennials and take two years to complete their life cycle before dying. Perennials live for many years and when they die it is usually because of an infection or some other environmental factor.

Apical meristems

Meristems are often differentiated based on their location within the plant. Apical meristematic tissue, sometimes referred to as primary meristem, occurs at the tips of roots and stems. The apical meristem and the surrounding developing tissue are known as the shoot apex. The shoot apex produces new tissue and causes primary growth through the process of mitosis and cell division (see Figure 9.17) Primary growth allows the root to extend throughout the soil. It also allows the stem to grow longer and so increases exposure to light and carbon dioxide. This type of growth results in herbaceous, non-woody stems and roots.

Lateral meristems

Lateral meristems allow the growth in thickness of plants. This is referred to as secondary growth (see Figure 9.17). Most trees and shrubs (woody plants) have active lateral meristems. In fact, these plants have two types of lateral meristem:

- vascular cambium, which produces secondary vascular tissue and lies between the xylem and the phloem in the vascular bundles, on the inside it produces secondary xylem, which is a major component of wood, and on the outside it produces secondary phloem
- cork cambium, which occurs within the bark of a plant and produces the cork cells of the outer bark.

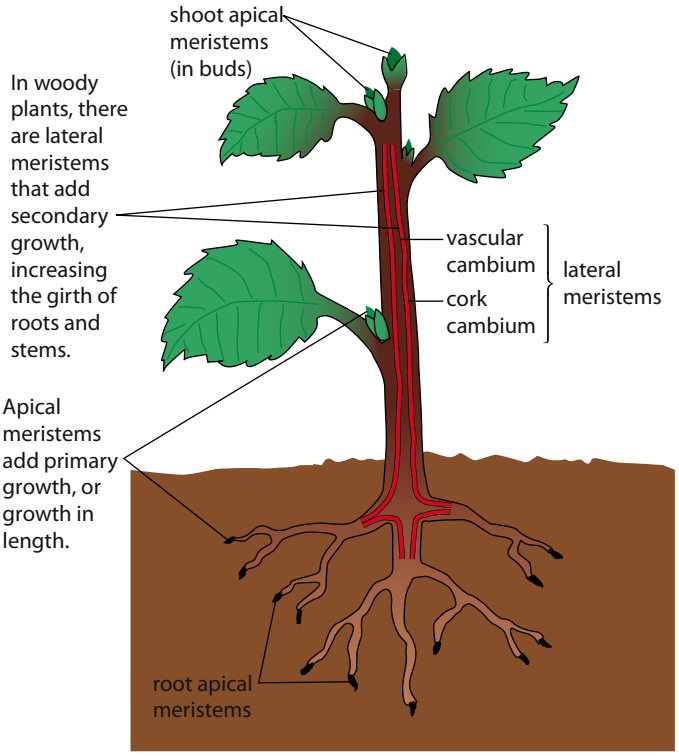


Figure 9.17 An overview of primary and secondary growth.

In seasonally growing perennial plants, annual rings are often formed as a result of secondary growth. These rings can be used to determine the age of the plant, as well as to determine the relative climate of the year of growth.

Plant hormones

Plant development involves extensive coordination amongst the individual cells that make up the plant. This requires a means of communication within the plant. Research has shown that there are many factors that affect plant development and growth. They include:

- environmental factors, such as day length and water availability
- receptors, which allow the plant to detect certain environmental factors
- the genetic makeup of the plant
- hormones, which are chemical messengers.

Specific cells have proteins (receptors) in their plasma membrane, cytoplasm, or nucleus that allow them to receive different environmental stimuli. Upon reception of a certain stimulus, the protein receptor becomes activated, initiating a metabolic pathway. This pathway often results in the production of a hormone (a chemical messenger). These hormones are produced in very small amounts and have effects in many parts of the plant. The hormones move in the plant through the phloem or from cell to cell. Cells on which a hormone has an effect are referred to as target cells.

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In 1880, Charles Darwin and his son Francis carried out some of the first experiments involving plant hormones. They described the effects of light on the movement of canary grass (*Phalaris canariensis*). The Darwins worked with the coleoptiles, the initial section of the stem during germination, of canary grass to find that they could bend the plant towards a unidirectional light source. In 1926, Fritz Went isolated the plant growth substance that the Darwins had been studying all those years before. For his isolation technique, Went used agar blocks. He even indirectly quantified the amount of growth substance in the agar blocks, by measuring actual curvatures of the stems. His results suggested that the curvature of stems was directly proportional to the amount of growth substance in the agar.

However, it is only recently that techniques have been developed to measure the extremely small amounts of the growth substance the Darwins and Went had experimented with. Another recent finding has allowed scientists to determine the effects of plant hormones on gene expression. Because of these developments, scientists are developing methods to provide greater plant growth and food production.

TOK

Research indicates a rather sophisticated communication abillity in plants. They have sophisticated systems for receiving information from their environment. Their responses to the environment increase their chance for survival as an organism and as a species. Do you believe plants possess a 'language' based on the information presented here?

Even though both plant and animal hormones act as chemical messengers in the organism, plant hormones differ from animal hormones in several ways. Plant hormones have varying effects depending on the receptor's location in the plant. In plants, unlike in animals, there is often a great deal of interaction between the different hormones to bring about the most appropriate response. In animals, it is common for one specialized gland or cell to produce a hormone. In plants a hormone may be produced throughout the plant.

The remainder of this section will focus on a group of common plant hormones called auxins.

Auxins and phototropism

Tropisms are generally defined as growth or movement to directional external stimuli. Tropisms may be positive (towards the stimulus) or negative (away from the stimulus). Common stimuli for plant tropisms include chemicals, gravity, touch, and light. Let's consider light as a stimulus. Phototropism means plant growth in response to light. Generally, plant stems exhibit positive phototropism, and plant roots demonstrate negative phototropism (see Figure 9.18). It is easy to demonstrate plant tropisms in the laboratory.

The leaves of plants grown inside will often show movement in the direction of the light they receive. To make sure a house plant maintains a more rounded appearance, it has to be turned regularly. This is an example of positive phototropism.

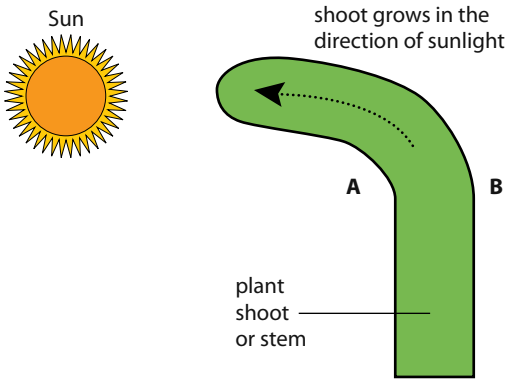

Figure 9.18 The effect of sunlight on a stem shoot. There is a higher concentration of auxin on side B, creating increased elongation of these cells and thus growth towards the light.

The importance of phototropism to a plant is clear. If an area is crowded with plants, it is essential for seedlings to grow towards the sunlight so that photosynthesis can occur efficiently. Auxins are plant hormones that cause the positive phototropism of plant shoots and seedlings.

Auxins are found in the embryo of seeds, the meristems of apical buds (shoot apex), and young leaves. These hormones only work on plant cells that have auxin receptors. Auxins appear to increase the flexibility of plant cell walls in young developing shoots. This enables cell elongation on the side of the shoot necessary to cause growth towards the light. This explains the response to light illustrated in Figure 9.18.

This growth response does not appear to be the result of an increased production of auxin on one side of the shoot. Rather, it seems to be caused by a redistribution of available auxin, especially to the side of the stem away from the light source. In the case of phototropism, auxin is actually produced in all the cells in this region of the plant. Auxin efflux pumps (specialized membrane proteins) move the auxins out of the cells closer to the light, using ATP as the energy source. This pumping action creates a high concentration of auxin in the space between the cells. The result is a high concentration of auxin in the intercellular space and a relatively low concentration within the adjacent cell. Because of this, auxin moves down the concentration gradient from the intercellular space into the adjacent cell. The entry of auxin into a cell is called auxin influx. This mechanism of auxin movement between adjacent cells continues until there is a greater concentration of auxin on the stem's dark side. The result is a greater elongation of cells on the stem side away from the light and, therefore, curvature towards the light source. The specific plant auxin that causes this described action is indoleacetic acid (IAA).

The elongation of the cells is caused by an expansion of the cell walls on the side away from the light source. The key step in this expansion is when auxin combines with a receptor that targets specific transcriptional repressors of auxin-responsive genes. The result of this altered gene expression is shown in Figure 9.19.

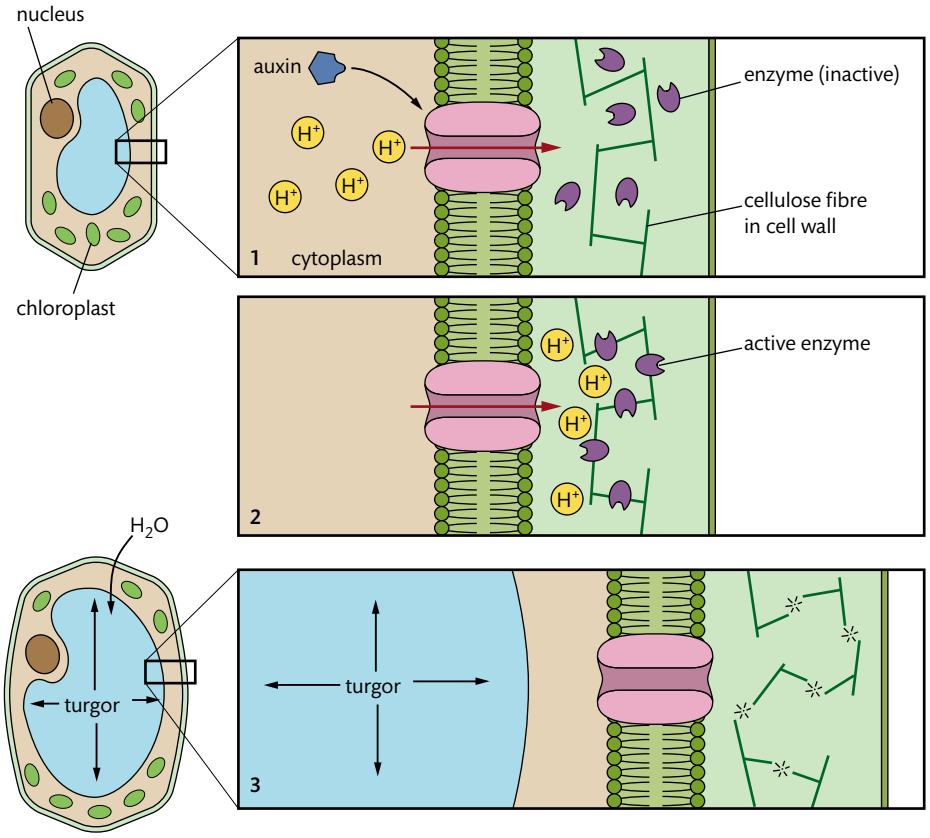

Figure 9.19 The mechanism that causes stems to bend towards light as a result of the influence of auxin.

The sequence of events in the stem causing it to bend towards a light source is as follows.

- Auxin is produced by all cells in the stem on the side towards the light source.
- Auxin moves by efflux pump action into the nuclei of cells on the side of the stem opposite the light.
- The auxin and a receptor in the nuclei form a complex that activates a proton (hydrogen ion) pump. Present research indicates that auxin itself also has an effect on the protein pump of the stem cell membranes as indicated in Figure 9.19.
- The proton pump moves hydrogen ions into the spaces of the cell wall.
- These hydrogen ions cause a drop in pH, resulting in the hydrogen bonds between cellulose fibres of the cell wall breaking.
- This results in the elongation of the cells on the side away from the light.

Auxins are a very complex set of hormones that have many functions within a plant. As well as the effect they have in phototropism, auxins are also involved in:

- stimulation of cell division in most meristematic tissue
- differentiation of xylem and phloem
- development of lateral roots in tissue cultures
- suppression of lateral bud growth when present in the apical bud
- stimulation of growth of flower parts
- induction of fruit production without pollination.

Whenever auxin affects cell growth, it does so by changing the pattern of gene expression. This usually involves an interaction with a repressor of a particular gene.

Many gardeners will induce lateral growth in plants by pinching off the apical bud. This removal of the apical bud allows the production of much more lateral growth, often resulting in more flowers and fruit. Some varieties of tomato have an increased yield if the apical bud is removed early in growth.

Micropropagation uses our understanding of plants, plant meristems, and hormones to stimulate existing plant material to produce large numbers of progeny (offspring). The culturing of cells from the shoot apex often involves the use of nutrient agar gels and growth hormones to achieve maximum growth and quantity of particular types of plants. Rare and endangered plant species may be maintained using micropropagation procedures. It is used in the case of orchids, which have very small seeds, to grow plants more reliably in sterile cultures. One problem with micropropagation, however, is that it is extremely expensive. It is also difficult to maintain the pathogen-free environments that are essential for culturing meristematic tissue (which is used for many types of micropropagation). Recently, micropropagation has been used to develop virus-free strains of existing plants. Another facet of this type of plant culturing is the ability to alter the genome of existing plants to make them more beneficial to humankind.

Exercises

7 If you removed the apical meristem from a typical plant, what would be the effect on further plant growth?

8 How does auxin alter gene expression when influencing cell growth rates?

9 What does it mean when plants are said to have indeterminate growth?

10 Apply the terms negative phototropic and positive phototropic to the root and stem of most plants.

11 What is the function of an auxin efflux pump in plant cells?

9.4 Reproduction in plants

Understandings:

- Flowering involves a change in gene expression in the shoot apex.
- The switch to flowering is a response to the length of light and dark periods in many plants.
- Success in plant reproduction depends on pollination, fertilization, and seed dispersal.
- Most flowering plants use mutualistic relationships with pollinators in sexual reproduction.

Applications and skills:

- Application: Methods used to induce short-day plants to flower out of season.
- Skill: Drawing internal structure of seeds.
- Skill: Drawing half-views of animal-pollinated flowers.
- Skill: Design of experiments to test hypotheses about factors affecting germination.

Guidance

- *Students should understand the differences between pollination, fertilization, and seed dispersal but are not required to know the details of each process.*
- *Flowering in so-called short-day plants such as chrysanthemums is stimulated by long nights rather than short days.*

A field of wild flowers in Texas, USA.

Variety in flowers

You only have to enter a flower shop or walk through a field to appreciate the tremendous variety and beauty of the reproductive structures of plants. Of course, we are talking about the flowers. The flower is the hardworking and very successful reproductive structure of angiosperms.

Flowers vary greatly in size. One of the smallest is the size of a sesame seed and occurs in the aquatic plant *Wolffia*. At the other end of the scale is the jungle flower *Rafflesia arnoldii*. This plant grows in Southeast Asia. When fully mature, it measures up to 0.9 m across and can weigh as much as 7 kg. Like many flowers, it depends on insects for pollination. When mature, this flower smells like rotting meat, thus attracting flies that transfer pollen from the male reproductive structures to the female structures.

Angiosperms

Any plant that has a flower is known as an angiosperm. Most flowering plants coevolved with pollinator species, such as insects, bats, and birds. These animals, as with the example of *Rafflesia*, facilitate the transfer of the male pollen to the female reproductive portions of flowers so that fertilization and seed development within the ovaries can occur.

For a long time, biologists have grouped angiosperms into two classes: the monocots (monocotyledonous plants) and the dicots (dicotyledonous plants). This division is based on morphological characteristics. Recently, new groupings have been emphasized because of a better understanding of the evolutionary development of the angiosperms. These new groupings involve analysis of DNA.

Table 9.4 shows differences in angiosperms based on morphological traits (see also Figure 9.20).

Table 9.4 A comparison of monocots and dicots

Monocots	Dicots
Parallel venation (the system of veins) in leaves	Netlike venation pattern in leaves
Three flower parts, or multiples of three	Four or five flower parts, or multiples of four or five
Seeds contain only one cotyledon (seed leaf)	Seeds contain two cotyledons (seed leaves)
Vascular bundles arranged throughout the stem	Vascular bundles arranged as a ring in the stem
Root system mainly fibrous	Root system involves a taproot (main root)
Pollen grain with one opening	Pollen grain with three openings

Based on phylogeny (evolutionary history), many biologists are now using three groups of angiosperms:

- the magnoliid complex (magnolias and laurels)
- the monocots
- the eudicots (true dicots).

This system is likely to change as more information is analysed.

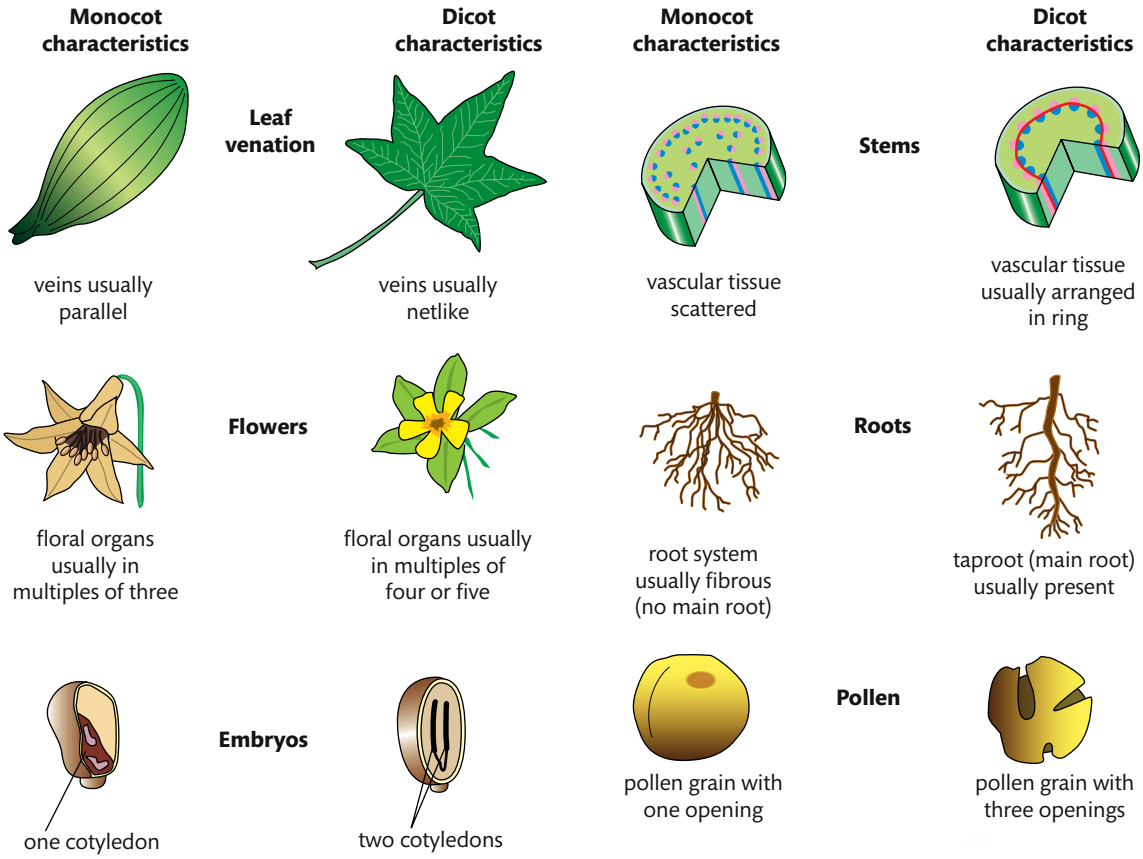

Figure 9.20 Compare these drawings to the table of features (Table 9.4) comparing monocots and dicots.

Flower structure and function

In both dicots and monocots, animal-pollinated flowers have the same parts in common. The flower parts and their functions are summarized in Table 9.5.

Table 9.5 A comparison of flower parts

Flower part	Function
Sepals	Protect the developing flower while it is inside the bud
Petals	Often colourful to attract pollinators
Anther	Part of the stamen that produces the male sex cells (pollen)
Filament	Stalk of the stamen that holds up the anther
Stigma	Sticky top of the carpel, on which pollen lands
Style	Structure of the carpel that supports the stigma
Ovary	Base of the carpel, in which the female sex cells develop

The entire female part of the flower is called the carpel. In some cases, the term pistil is used to refer to a single carpel or a group of fused carpels. The entire male part of the flower is called the stamen.

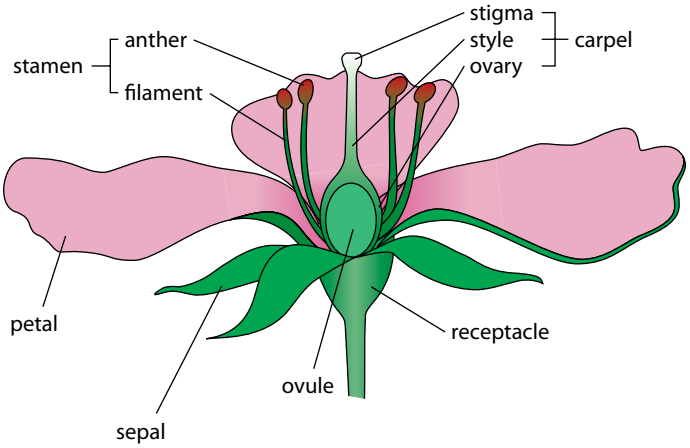

Figure 9.21 An animal-pollinated dicotyledonous flower typically shows these structures.

Flowers occur in a myriad of colours, shapes, and types. Some different types are:

- complete flowers, which contain all four basic flower parts, the sepals, petals, stamen, and carpel
- incomplete flowers, which lack at least one of the four basic parts
- staminate flowers, which only have stamens, and no carpels
- carpellate flowers, which only have carpels, and no stamens.

Meiosis occurs in the stamen and carpel to produce the sex cells.

Pollination and fertilization

All plants show two different generations in their life cycle. The generations are:

- the gametophyte generation, which is haploid
- the sporophyte generation, which is diploid.

In plants, these two generations alternate with one another. Not surprisingly, this is called alternation of generations. The generations are named according to the reproductive cells they produce. The gametophyte generation produces the plant gametes by mitosis, whereas the sporophyte generation produces spores by meiosis. When we look at a flowering plant such as a cherry tree, we are looking at the sporophyte generation. It grew from a zygote and produces new cells by mitosis. When the cherry tree produces flowers, haploid spores are formed and develop into the haploid bodies referred to as gametophytes. Sperm form within the male gametophytes, and eggs form within the female gametophytes.

Pollination and fertilization are two very different processes in plants. Let's consider pollination first.

The University of Göttingen in Germany found in recent studies that nearly 76% of the world's leading crops are pollinated by animals. Bees account for a significant percentage of plant pollination brought about by animals. Most of these plants are extremely important in providing food for the world's people. The problem we are now facing is that the world bee population seems to be in decline. Possible reasons for this decline include widespread use of pesticides, deforestation and destruction of colony habitats, and pathogens. It is extremely important that research be carried out to determine the cause(s) of this population decrease. If not, the decline in food production could be devastating to the human population.

Allergic rhinitis, commonly referred to as hay fever, is a distressing condition that is both common and widespread throughout the world. It is often caused by pollen in the air. People with this condition have an immune response to the proteins that project from the outer surface of the pollen.

The pollen that causes hay fever is usually from wind-pollinated plants and is, therefore, very light. Pollen from insect-attracting flowers is relatively heavy and is unlikely to be in the air and to be a cause of allergic rhinitis. Currently, very little pollen of either type is found in desert and polar regions of the world.

Pollination

Pollination is the process by which pollen (containing male sex cells) is placed on the female stigma. It is the first step in the progression towards fertilization and the production of seeds. Pollen can be carried from anther to stigma by a variety of means. The earliest seed plants relied upon wind as their pollen vector. Later, insects became a major factor in the process. It appears that the first angiosperms were pollinated by insects. There is very convincing fossil evidence showing that the angiosperms and insects coevolved; they appear to be instrumental in each other's development.

There are many vectors of pollination besides insects and wind. These include birds, water, and animals other than insects. Most flowering plants use mutualistic relationships with pollinators in sexual reproduction. Flowers of plants that involve insect or other animal pollinators employ various means to attract their vector. For example:

- red flowers are conspicuous to birds
- yellow and orange flowers are noticed by bees
- heavily scented flowers can be located at night.

Plants that rely on wind as their pollen vector have inconspicuous, odourless flower parts.

There are two general types of pollination:

- self-pollination
- cross-pollination.

In self-pollination, pollen from the anther of the same plant falls upon its own stigma. Self-pollination is a form of inbreeding and results in less genetic variation within a species.

When cross-fertilization occurs, pollen is carried from the anther of one plant to the stigma of a different plant of the same species. Cross-pollination increases variation and may result in offspring with better fitness. The problem with cross-pollination is that the female stigma may not receive the male pollen because of the longer distance to travel.

Botanists can select plant genetic characteristics by controlling the process of pollination. Gregor Mendel controlled the process of pollination in garden pea plants in the development of his genetic principles.

Once pollination occurs, the next step is fertilization.

Fertilization

Fertilization happens when the male and female sex cells unite to form a diploid zygote. The female sex cells that are fertilized by the pollen are present within the ovules of the flower. The ovules are present within the ovary of the carpel. When the pollen grain adheres to the stigma, which is covered by a sticky, sugary substance, it begins to grow a pollen tube. Pollen tube growth and fertilization occur in the following sequence.

- Pollen germinates to produce a pollen tube.
- The pollen tube grows down the style of the carpel.
- Within the growing pollen tube is the nucleus that will produce the sperm.
- The pollen tube completes its growth by entering an opening at the bottom of the ovary.
- The sperm moves from the tube to combine with the egg of the ovule to form a zygote.

Grasses, a type of monocotyledonous plant, have tiny, almost inconspicuous flowers. As these common plants do not have showy petals to attract insects, they rely on the wind for pollination. A fruit is a structure that originates from the ovary of a flower. Grass fruits, differing from most fruits by not containing seeds, are specifically called grain and they supply food for almost every human in the world. Specific examples of important grain crops include wheat, rice, and maize.

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A paradigm shift has occurred in our approach to saving the world's pollinators. Recognizing that human activity has placed a large pressure on pollinators by removing their natural habitats, and that pollinators prefer a diverse population of plants, scientists are now working to bring about the preservation of pristine natural vegetation and whole ecosystems. Prior to this, scientists had worked to protect individual plant species to save specific pollinators.

Fertilization in flowering plants is actually a double fertilization. After pollination, one of the two sperms produced by the pollen grain combines with the egg. The other combines with two polar nuclei within the ovary to produce a triploid (3*n*) endosperm. The endosperm has the function of storing nutrients for the young plant.

Once the zygote is formed it develops with the surrounding tissue into the seed. As the seed is developing, the ovary around the ovule matures into a fruit. The fruit encloses, and helps to protect, the seed.

The seed

The seed is the means by which an embryo can be dispersed to distant locations. It is a protective structure for the embryo. Seeds of dicotyledonous plants usually contain the parts shown in Figure 9.22. The seed parts and their functions are summarized in Table 9.6.

Table 9.6 Seed parts and their functions

Seed part	Function
Testa	A tough, protective outer coat
Cotyledons	Seed leaves that function as nutrient storage structures
Micropyle	A scar at the opening where the pollen tube entered the ovule
Embryo root and embryo shoot	Become the new plant when germination occurs

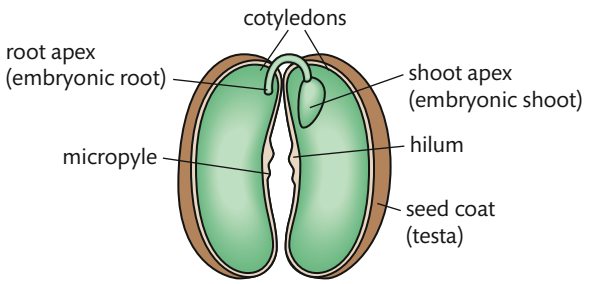


Figure 9.22 A dicotyledonous seed typically shows these structures. Note the two large cotyledons or seed leaves.

Seed dispersal is important in plant reproduction for a number of reasons. By moving away from the parent plant, the potential new plant faces a reduction in competition for limited resources. Fruits are mature ovaries (carpels) that contain seeds. Fruits have a variety of adaptations that allow successful dispersal of seed by wind, water, and animals.

In 1995, a team of biologists in China found some seeds in a dried-up lakebed. The seeds were from a type of lotus plant. After germinating some of the seeds, the biologists found them to be nearly 1300 years old. They used radiometric dating to determine this age.

Once seeds are formed, a maturation process follows. This process involves dehydration until the water content of the seed is about 10–15% of its weight. At this point, the seed usually goes into a dormancy period. This is a time of very low metabolism and no growth or development. The dormancy period is quite variable for different types of seeds. This represents an adaptation feature to overcome harsh environmental conditions.

If conditions become favourable, the seed will germinate. Germination is the development of the seed into a functional plant. There are several general conditions that must be present for a seed to germinate:

- water is needed, to rehydrate the dried seed tissues
- oxygen is needed, to allow aerobic respiration to produce ATP
- an appropriate temperature for the seed is necessary (temperature is important for enzyme action).

Besides these general conditions, many plants have specific conditions that must be met in order to germinate. For example, in some seeds the testa must be disrupted or scarified (broken) before water uptake can occur. Other seeds must be exposed to fire

or smoke before they germinate. The food product known as liquid smoke can cause seeds of this type to germinate. Liquid smoke is produced when smoke from certain types of burning wood is allowed to condense in water. It can be purchased from several online sources and in many large food stores. Light is generally not mentioned in discussions about seed germination because it has variable effects on the process.

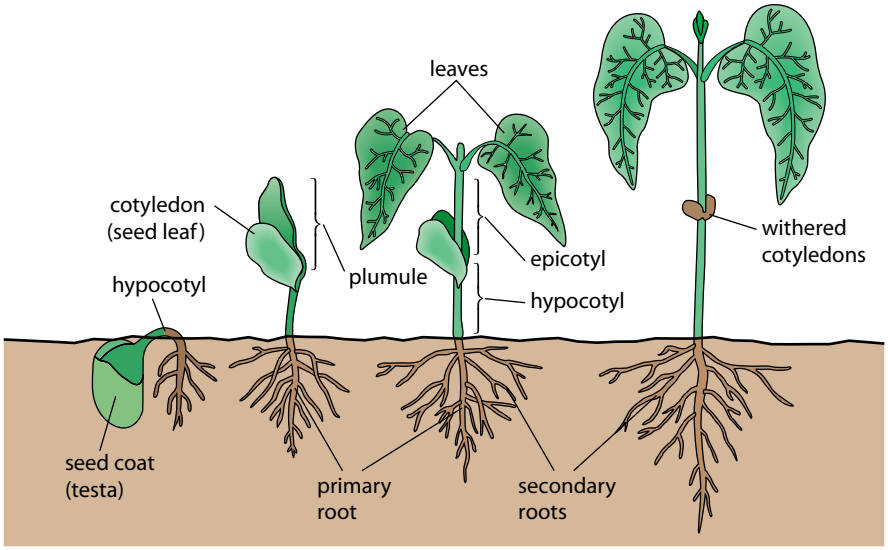


Figure 9.23 Stages in the germination of a bean seed, *Phaseolus vulgaris*. The plumule is the shot tip and includes the epicotyl and its two developing leaves. The epicotyl is the region above the attachment point of the cotyledons to the stem. The hypocotyl is the region immediately below the cotyledon attachment point.

Seed germination is an uncertain time in a plant's life. The emerging seedling is fragile and it will be exposed to harsh weather, parasites, predators, and many other hazards.

It is essential that you are able to draw the internal structure of seeds. Dissection of various types of seeds in a laboratory setting, as well as studying seed diagrams, will be a great help in developing this skill. Various species of bean seeds are ideal for this study.

The initial processes in the germination of a seed are:

- the absorption of water
- release of a plant growth hormone called gibberellin or gibberellic acid
- gibberellin causes production of amylase, which hydrolyses starch into maltose
- maltose is further hydrolysed into glucose, which can be used in cellular respiration
- the early glucose may also be converted into cellulose so that the cell walls of new cells may be produced.

Factors affecting seed germination

Safety alerts: Wear protective eyewear and an apron. Be cautious in the use of the fungicide. Follow your teacher's directions carefully concerning the use and disposal of the fungicide. If mould, (fungal) growth becomes visible, discard the setup according to your teacher's instructions. Caution: mould may cause allergic reactions in some people. Wash your hands thoroughly after working in any phase of this procedure.

There are many factors that can affect germination. In this activity, you will research these factors and formulate a hypothesis concerning how a specific factor affects germination of a particular type of seed. Once you have produced your hypothesis, design an experiment to test it. Many types of seeds may be used, and you will encounter several suggestions in your research.

It is advisable to dip the seeds in a fungicide, such as 5% bleach, for 15 minutes before carrying out your procedure. This step will decrease bacterial and/or mould growth.

There are many methods for carrying out seed germination experiments. Choose one that is most suited to your circumstances.

Once you have carried out your procedure, determine whether your data verify your hypothesis. Evaluate the validity of your procedure. Share your results and discuss your findings with your classmates.

Many of the seeds will not produce a functional plant because of these dangers. To compensate, plants produce large numbers of seeds so that the species can survive.

The control of flowering in angiosperms

Light is a very important factor in the life of a plant. It is required for photosynthesis, and it controls many aspects of plant growth and development. Plants are able to detect the presence of light, its direction, wavelength, and even intensity. Photoperiodism is the plant's response to light involving the relative lengths of day and night: a very important factor in the control of flowering. To ensure continued existence in an area, a plant must flower when pollinators are available and when necessary resources are plentiful. Table 9.7 summarizes three categories of plant in relation to light and flowering.

Table 9.7 Types of flowering in angiosperms

Plant type	Flowering and light	Examples
Long-day plants	Flower when days are longest and nights are shortest (midsummer)	Radishes, spinach, and lettuce
Short-day plants	Flower in spring, late summer, and autumn, when days are shorter	Poinsettias, chrysanthemums, and asters
Day-neutral plants	Flower without regard to day length	Roses, dandelions, and tomatoes

Euphorbia pulcherrima, commonly known as poinsettia, requires at least 14 hours of darkness, uninterrupted by light, in order to produce the red-coloured displays they are famous for. In its natural setting, the appropriate length of darkness occurs towards the winter season. Commercially, these plants are now grown in huge greenhouses where the amount of sunlight is controlled to cause flowering at any time of year. In fact the red-coloured parts of the plant that develop are not the flowers. They are specialized leaves called bracts that develop at the same time as the plant's tiny flowers; the tiny flowers are at the centre of a group of bracts.

Even though the names refer to day length, it is actually the length of night that controls the flowering process in plants of the long-day and short-day types. This was shown in the 1940s with experiments that interrupted periods of darkness with brief exposures to light.

Chrysanthemums are an example of short-day (long-night) plants. To initiate flowering in these plants, some growers will place a black cloth over a plant for 12–15 hours a day until the flower buds begin to show colour.

The control by light is brought about by a special blue-green pigment in the plants called phytochrome. There are two forms of phytochrome. One form is inactive and is represented by P_r. The other is active and is represented by P_{fr}.

When red light (which has a wavelength of 660 nm) is present in available light, the inactive form of phytochrome, P_r, is converted to the active form, P_{fr}. This conversion occurs rapidly. P_{fr} has the ability to absorb far-red light (which has a wavelength of

730 nm). This P_{fr} is rapidly converted back to P_r in daylight. However, in darkness the conversion back to P_r is very slow. It is thought that it is this slow conversion of P_{fr} back to P_r that allows the plant to time the dark period. This seems to be the controlling factor for flowering in short-day and long-day plants.

In long-day plants the remaining P_{fr} at the end of a short night stimulates the plant to flower. In other words, it acts as a promoter in these plants. However, in short-day plants the P_{fr} appears to act as an inhibitor of flowering. For these short-day plants, enough P_{fr} has to be converted to P_r for flowering to occur.

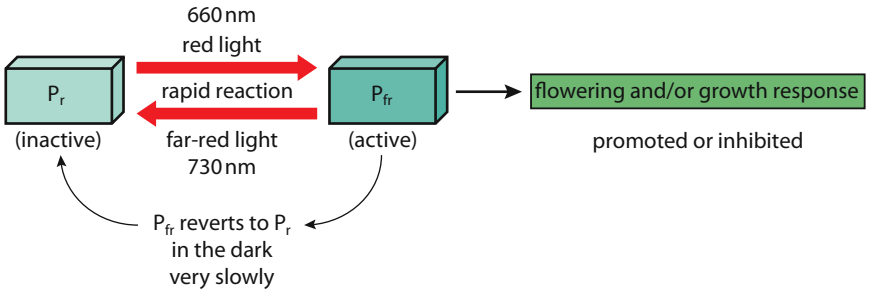


Figure 9.24 Interconversion of the phytochrome molecule between its two possible forms. This mechanism promotes or inhibits flowering in certain plants.

P_{fr} is able to stimulate flowering by activating specific genes of the shoot apex cells in a plant. This activation results in changes in DNA transcription (gene expression), thus allowing the production of flowers.

Arabidopsis thaliana is a small flowering plant native to Europe and Asia that has been studied extensively regarding light sensing and flower development. Research has demonstrated that 10% of the plant's entire genome (2500 genes) has altered transcription factors when flowering occurs.

Exercises

12 What features would a seed possess that is capable of being successfully dispersed by wind?

13 Why is oxygen important for the germination of seeds?

14 The micropyle allows water to enter the testa of a seed during germination. What was the significance of the micropyle to the ovule?

15 Why is it possible to purchase a chrysanthemum in full bloom at any time of the year when it is a short-day plant?

16 Suggest some reasons why pollinators are in trouble worldwide.

Practice questions

1 Plants develop brightly coloured flowers to attract animals. Which process is directly assisted by this adaptation?

A Seed dispersal
B Pollination
C Fertilization
D Germination

(*Total 1 mark*)

2 How are fluids transported in the xylem and the phloem?

	Xylem	Phloem
A	away from the root only	towards the root only
B	towards the root only	away from the root only
C	away from and towards the root	towards the root only
D	away from the root only	away from and towards the root

(*Total 1 mark*)

3 What controls the flowering process in long-day plants?

A P_{fr} is converted by red light to P_r, which acts as a promoter of flowering.
B P_r is converted by red light to P_{fr}, which acts as an inhibitor of flowering.
C P_r is converted by red light to P_{fr}, which acts as a promoter of flowering.
D P_{fr} is converted by red light to P_r, which acts as an inhibitor of flowering.

(*Total 1 mark*)

4 What generates new cells in dicotyledonous plants?

I Apical meristems II Lateral meristems III Phloem

A I only.
B II only.
C I and II only.
D I, II, and III.

(*Total 1 mark*)

5 **(a)** Outline the thermal, cohesive, and solvent properties of water. (5)

(b) Outline adaptations of xerophytes. (4)

(*Total 9 marks*)

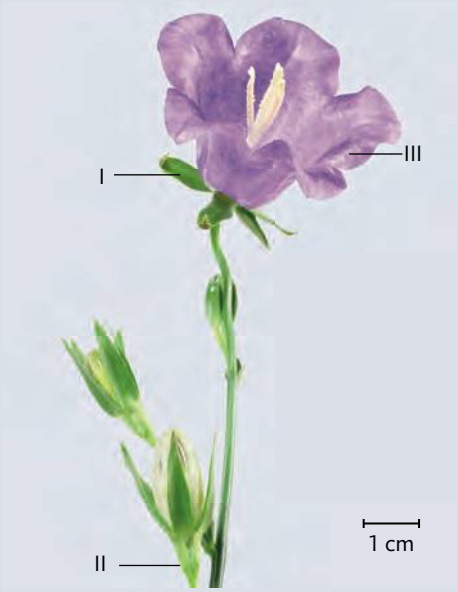
6 **(a)** Describe how water is carried by the transpiration stream. (7)

(b) Explain how flowering is controlled in long-day and short-day plants. (7)

(Plus up to 2 for quality)

(*Total 16 marks*)

7 **(a)** The photograph below shows the flowers of *Campanula persicifolia*.
Label structures I, II and III.



(3)

(b) **(i)** Using the external features shown in the photograph, state the phylum to which this plant belongs. (1)

(ii) Comment on the hypothesis that the plant shown in the photograph could be pollinated by an animal. (2)

(*Total 6 marks*)

10 Genetics and evolution

Essential ideas

10.1 Meiosis leads to independent assortment of chromosomes and unique composition of alleles in daughter cells.

10.2 Genes may be linked or unlinked and are inherited accordingly.

10.3 Gene pools change over time.

Hummingbirds show impressive adaptation to their habitat. Those birds who possess the genes for bills long enough to drink nectar at the bottom of long, thin flowers have a better chance of survival than those who do not. The same can be said concerning the genes allowing them to hover and even fly backwards.

What is the secret? How is it that each organism produced by the fusion of two gametes has its own genetic makeup? How is it possible that each insect, each child, each goldfish, each tree, is unique, among thousands, millions or even billions? This chapter will explore how genes are shuffled to make new combinations, and not only how unique individuals are produced but also how new species evolve.

10.1 Meiosis

Understandings:

- Chromosomes replicate in interphase before meiosis.
- Crossing over is the exchange of DNA material between non-sister homologous chromatids.
- Crossing over produces new combinations of alleles on the chromosomes of the haploid cells.
- Chiasmata formation between non-sister chromatids in a bivalent can result in an exchange of alleles.
- Homologous chromosomes separate in meiosis I.
- Sister chromatids separate in meiosis II.
- Independent assortment of genes is due to the random orientation of pairs of homologous chromosomes in meiosis I.

Applications and skills:

- Skill: Drawing diagrams to show chiasmata formed by crossing over.

Guidance

- *Diagrams of chiasmata should show sister chromatids still closely aligned, except at the point where crossing over occurred and a chiasma was formed.*

NATURE OF SCIENCE

Making careful observations: careful observation and record keeping turned up anomalous data that Mendel's law of independent assortment could not account for. Thomas Hunt Morgan developed the notion of linked genes to account for the anomalies.

Although these apples may look similar, inside they are carrying seeds that each has a unique combination of genes. This ensures that some will have a winning combination and could become successful new trees.

Multiple genes

What happens to chromosomes during the formation of gametes so that offspring are always different? Even if your parents had dozens of other children, none would be identical to you.

Chapter 3 looked at examples of genetics with single genes that have two alleles (dominant or recessive), such as wet or dry earwax, or single genes with multiple alleles, such as blood type (which can be used to demonstrate co-dominance). The idea of sex-linked genes for colour blindness and haemophilia was also explored.

But what if two or five or ten genes controlled a single trait? Could the many alleles all work together to contribute to the trait, in the way each instrument in an orchestra contributes to a symphony? Instead of having either one characteristic or another, could there be a range of traits from one extreme to another? This section will look at these issues.

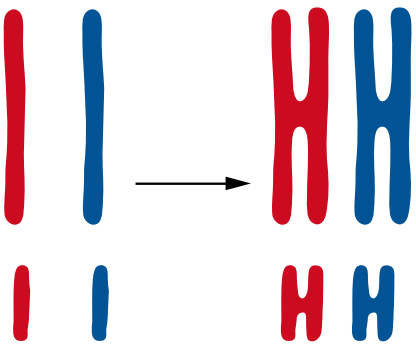


Figure 10.1 Two pairs of single chromosomes being replicated. After replication during interphase, there are still two pairs of chromosomes, the difference is that now they have two full copies of each chromosome connected at the centromere.

Chromosome replication

Before meiosis can occur, the cell must prepare for cell division during interphase. An important step in this preparation is DNA replication, enabling the cell to have a copy of each chromosome.

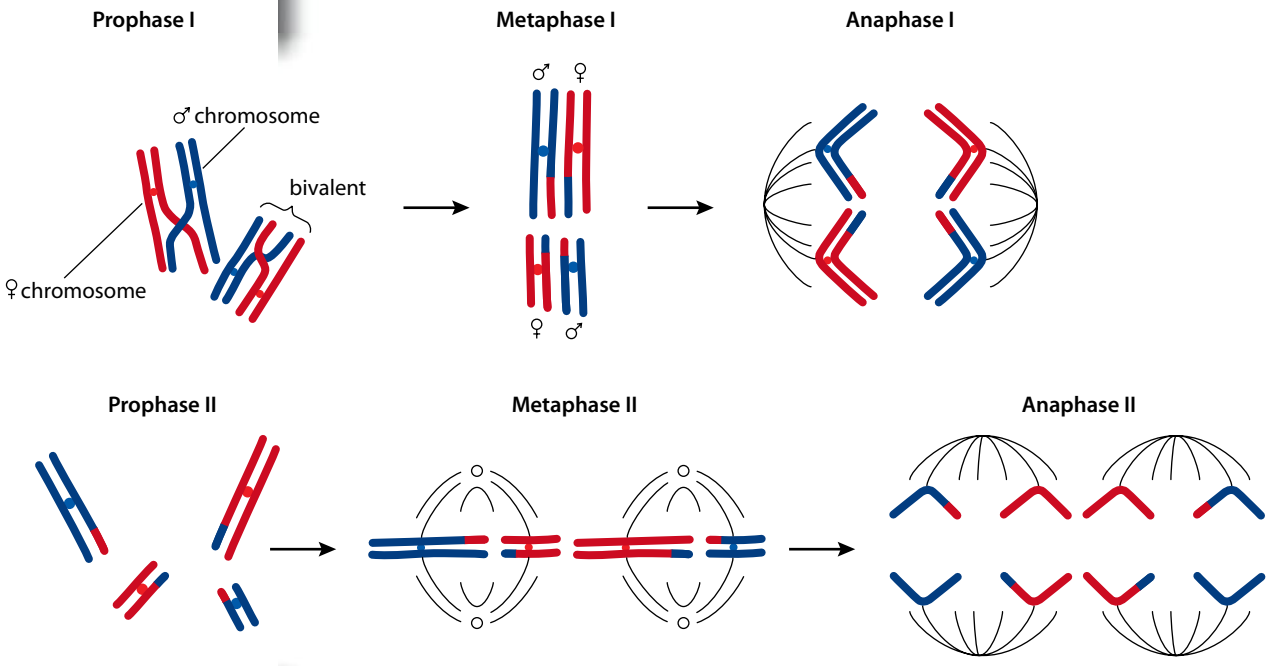


Figure 10.2 Some of the crucial steps in meiosis. Telophase I and II are not shown.

The exchange of DNA material

During prophase I of meiosis (see Figure 10.2), the process of synapsis brings together two homologous chromosomes in a pair called a bivalent. Recall that the term homologous means that the chromosomes are the same length, they have their centromeres in the same position, and generally they contain the same genes stored at the same loci. The major difference between them is that one chromosome in the bivalent came from the person's mother and the other chromosome in the bivalent came from the person's father. As each parent can have different alleles for each of the genes along the chromatids, the two homologous chromosomes are by no means identical. On the other hand, recall that the two sister chromatids from the same chromosome should be identical because they are the result of DNA replication during interphase.

Mixing genetic material between non-sister chromatids, in other words between paternal and maternal chromosomes, can occur when the chromatids intertwine and break. In order for crossing over to function correctly, identical breaks must occur at exactly the same position in adjacent non-sister chromatids (see Figure 10.3)

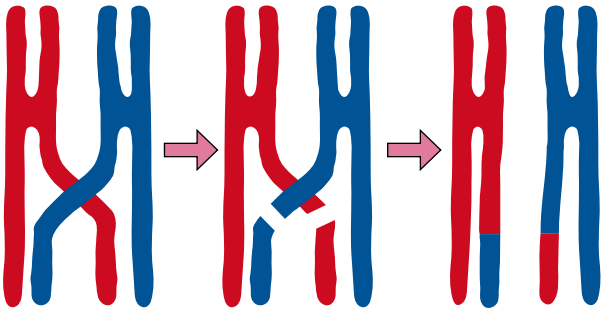
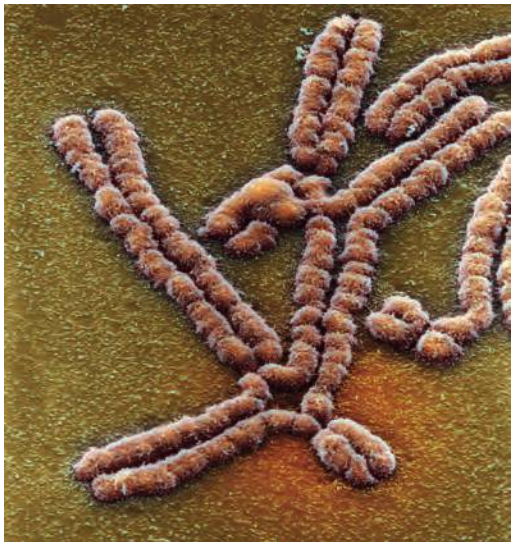


Figure 10.3 Crossing over between non-sister chromatids.

New combinations of alleles

For crossing over to happen, each chromatid involved has to have a separated tip. The two segments each connect to the corresponding position on the other chromatid. The two tips are thus switched and each resulting chromatid has a segment of the other's genetic material. The place where the two connect to each other is called a chiasma (plural chiasmata). For simplicity, Figure 10.3 shows only one chiasma, but in reality many can form along all four chromatids.

Once they are attached at their chiasmata, the chromatids repel each other and twist around to make interesting shapes, depending on where and how many times they are attached (examples are shown in the photo).



This false-colour scanning electron micrograph (SEM) shows the cross-shaped union of chromosomes exchanging material, which is where the name chiasma (meaning cross) comes from.

Chiasmata formation

As we have seen, the process of crossing over happens when two non-sister chromatids swap segments of their DNA. This means that a maternal chromosome can end up with a segment of genetic material from a paternal chromosome, and vice versa. Thus a chromosome originally carrying a recessive allele could end up with a dominant allele that was traded during crossing over.

For example, consider a bivalent in which the maternal chromosome has allele **B** for an autosomal trait, and the paternal chromosome has the recessive allele **b**. Figure 10.4 shows a chiasma between such a gene's locus and the centromere. Recall from Section 3.2 (Figure 3.11) that autosomal means that the gene causing the trait is found on one of the 22 chromosomes that are not the sex chromosomes.

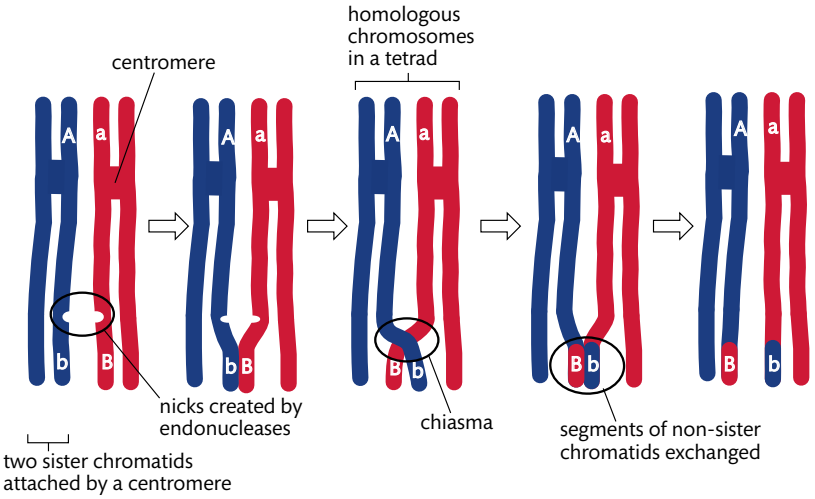


Figure 10.4 How an allele **B** from a maternal chromosome (in red) can be switched with an allele **b** on a paternal chromosome (in blue).

The main ways in which gamete production is able to generate genetic variety in offspring are:

- **crossing over during prophase I**
- **random orientation during metaphase I.**

When crossing over is complete, the segments containing the locus of the gene have been swapped and the alleles have switched places. Now it is the 'paternal' chromosome (no longer 100% paternal) that has **B** and the 'maternal' chromosome that has **b**. Note that the two paternal sister chromatids no longer carry identical alleles. Remember to say **B** as 'big B' and **b** as 'little b'.

During any single crossing over event, hundreds or thousands of genes can be traded in this way between non-sister chromatids. In addition, a single bivalent can have several chiasmata producing crossing over in more than one chromatid. This is yet another source of variation in the formation of sperm cells and egg cells. This also partially explains why, unless they are identical twins, brothers and sisters never get the same combination of their parents' alleles.

CHALLENGE YOURSELF

1 Using Figure 10.4 as a template, make an annotated biological drawing including the following:

- two homologous chromosomes aligned, being sure to show different alleles of the genes on these chromosomes
- nicks made by endonucleases where the chromatids will start to bend
- the chiasma being formed
- tips of chromatids being exchanged
- be sure to include a title
- use shading or cross-hatching in addition to annotations to show where crossing over is taking place.

Homologous chromosome separation

As seen in Figure 10.5, the homologous chromosomes are pulled to opposite ends of the cell during meiosis I, notably visible during anaphase I. Figure 10.5 does not show telophase I, which involves the chromosomes unravelling and two cells forming.

Notice the difference between anaphase I of meiosis and anaphase during mitosis: instead of the sister chromatids of a single replicated chromosome being pulled apart the way they are in mitosis, the bivalents in meiosis are separated so that each homologous chromosome is pulled to opposite ends of the cell.

Again, although they are similar, homologous chromosomes are not identical to each other. They carry the same genes but not necessarily the same alleles and, by now, they would have swapped some alleles during crossing over.

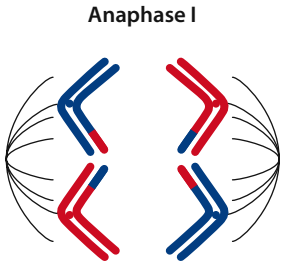


Figure 10.5 Anaphase I, during which homologous chromosomes (bivalents) are separated. Notice the swapped tips of chromosomes where crossing over has happened.

Sister chromatid separation

It is not until meiosis II that the sister chromatids of each chromosome are separated. As can be seen in Figure 10.6, in anaphase II the centromeres of the chromosomes split, releasing each sister chromatid to become an individual chromosome. The spindle microtubules pull individual chromatids to opposite ends of the cell. Because of random orientation, the chromatids can be pulled towards either of the newly forming daughter cells.

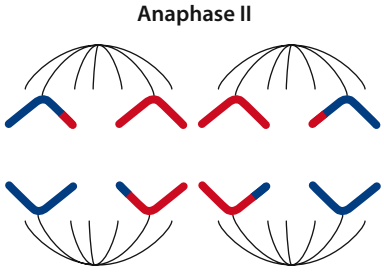


Figure 10.6 Anaphase II, during which sister chromatids are pulled to opposite poles of the cells.

During telophase II, a new nuclear membrane can form around each of the four new cells. Each of the four cells has a different combination of half the genetic material of the original parent cell.

First, when referring to meiosis, be sure to keep track of the differences between homologous chromosomes and sister chromatids. Second, do not forget that, when the sister chromatids are pulled apart, they become individual chromosomes ready to inhabit their newly forming cells. Also, keep in mind the importance of the steps in terms of shuffling genetic information in order to promote variety in the offspring.

Recall the reason why each cell needs to have only 50% of the parents' DNA: it is to enable gametes that meet with another gamete to form the next generation: 50% + 50% = 100% Although there are exceptions, notably with plants, generally it is not advantageous to have more than 100% of the DNA needed to make the next generation.

Independent assortment of genes

Gregor Mendel's law of independent assortment states that, when gametes are formed, the separation of one pair of alleles between the daughter cells is independent of the separation of another pair of alleles. What this means is that, as a general rule, one allele does not follow another when it is passed on to a gamete. The law of independent assortment implies that alleles that determine different characteristics will be transmitted independently to the next generation.

In practice, this means that, just because one trait (such as a certain flower colour) is inherited from a parent, it does not follow that any other specific trait of that parent (such as a specific seed colour) is passed on as well. On the contrary, each allele in a pair can mix with either allele of another pair.

As with many rules, there are exceptions. Some genes do, in fact, go hand-in-hand, so that when one is placed in a gamete during meiosis the other follows. We will explore such linked genes later in this chapter.

Independent assortment and meiosis

When Mendel was performing his experiments in the mid-1800s, he did not know anything about meiosis because it had not yet been discovered. Today, it is possible to answer the question that was most probably on his mind: Why do traits get passed on independently from each other?

The answer lies in an understanding of the process of meiosis. You will recall that the orientation of bivalents during metaphase I is a random process. To illustrate this, consider a cell with four pairs of chromosomes (see Figure 10.7). In each of the four bivalents, there is a maternal chromosome (red) and a paternal chromosome (blue). For simplicity, no crossing over is shown. Figure 10.7 shows three possibilities for random orientation:

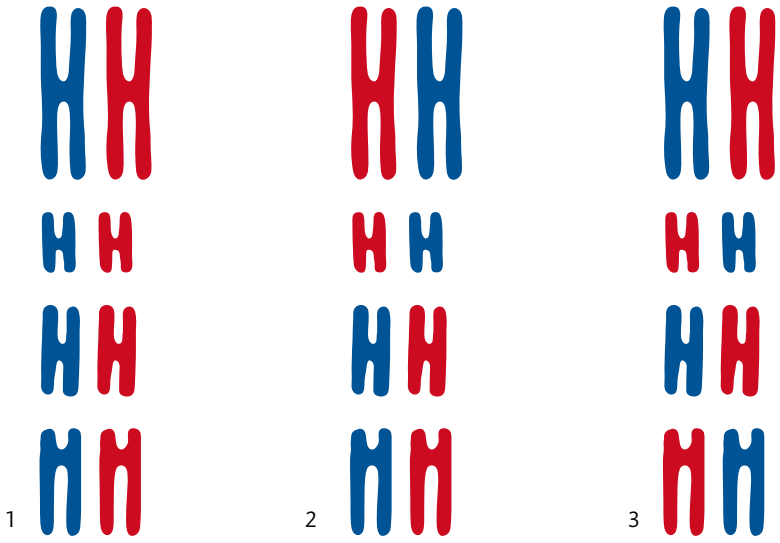


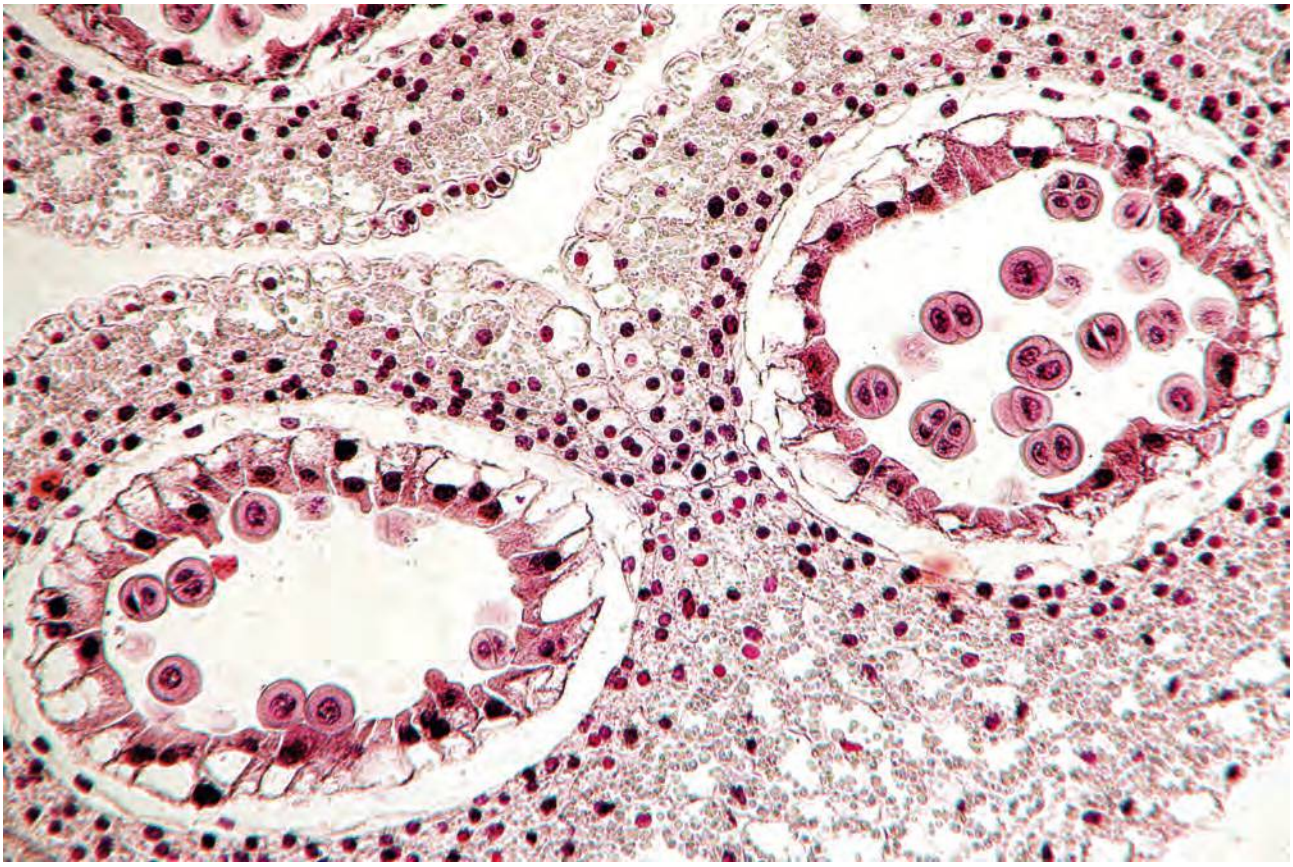
Figure 10.7 Three of the 16 possible orientations for four bivalents. In humans there are 23 bivalents with more than 8 million possible orientations.

For humans, the total theoretical number of possible combinations during random orientation is 2^{23} because there are 23 chromosomes in each gamete. Hence the probability that a woman could produce the same egg twice is 1 in 2^{23} or 1 in 8 388 608. This calculation is an oversimplification, however, because it does not take into consideration the additional variety that results from the process of crossing over.

In addition, the calculation only considers the mathematical probability in the production of one gamete. To produce offspring, two gametes are needed, and the chances that both parents produce two identical offspring (apart from identical twins) is infinitesimal.

In some ways, meiosis is like a lottery.

Inside a lily flower, the anthers produce pollen cells by meiosis. This photo through a light microscope shows a section through anthers with the pollen in various stages of cell division. Notice that some have formed four daughter cells ready to be used in fertilization. The lab investigation in this section explains how to see this for yourself.



Observing the stages of meiosis

Safety alerts: Attention: sharp utensils. Use caution when cutting the anther. Attention: acid. Acetocarmine contains acid and should not get on the skin or in the eyes. Wear eye protection and protect skin and clothing.

For this investigation, first place a drop of acetocarmine stain on an empty microscope slide. Then collect one anther from a lily flower and place it in the drop. Using a scalpel or dissecting needle, slice up the anther into sections that are as thin as possible (see Figure 10.8). Keep two or three of the thinnest slices flat on the slide in the drop of stain and remove the rest of the anther. Place a cover slip gently on the slices without crushing them. Observe the cells and identify the stages of meiosis.

Optional: if your lab has some available, observe slides that have been prepared by professionals. Also, you could try other types of germ-line cells if they are available. The term germ line refers to cells involved in passing genes on to the next generation.

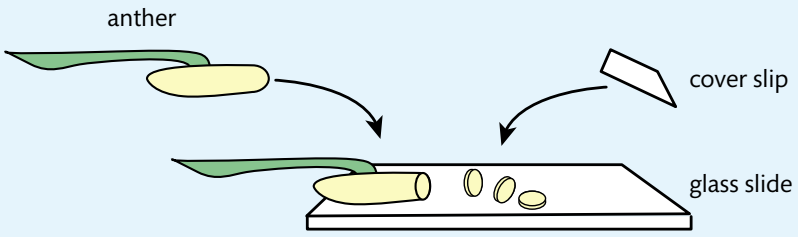


Figure 10.8 The part of the flower to prepare is a cross-section of the pollen sacs.

Exercises

1 State the phase of meiosis during which each of the following events happen.

(a) Sister chromatids are pulled to opposite ends of the cells.
(b) Pairs of homologous chromosomes line up along the equator of the cell.
(c) The formation of chiasmata occurs.
(d) Homologous chromosomes are separated and pulled to opposite sides of the cell.

2 What is the purpose of meiosis, and why is it so important to produce variety in the daughter cells that are produced by meiosis?

3 State Mendel's law of independent assortment. State one example of an exception to this law.

This section has shown multiple ways in which nature tries to increase variety in populations, and we have seen the power of variation in natural selection. Despite our understanding of the importance of variation for natural selection, biodiversity, and for our health, countries all over the world are reducing the variety of crops they plant, and are replacing balanced ecosystems with monocultures. Are such practices sustainable? Could reducing variety in the foods we grow and eat increase risks of famine and other global health crises?

10.2 Inheritance

Understandings:

- Gene loci are said to be linked if on the same chromosome.
- Unlinked genes segregate independently as a result of meiosis.
- Variation can be discrete or continuous.
- The phenotypes of polygenic characteristics tend to show continuous variation.
- Chi-squared tests are used to determine whether the difference between an observed and expected frequency distribution is statistically significant.

Applications and skills:

- Application: Morgan's discovery of non-Mendelian ratios in *Drosophila*.
- Application: Completion and analysis of Punnett squares for dihybrid traits.
- Application: Polygenic traits such as human height may also be influenced by environmental factors.
- Skill: Calculation of the predicted genotypic and phenotypic ratio of offspring of dihybrid crosses involving unlinked autosomal genes.
- Skill: Identification of recombinants in crosses involving two linked genes.
- Skill: Use of a chi-squared test on data from dihybrid crosses.

Guidance

- *Alleles are usually shown side by side in dihybrid crosses, for example TtBb. In representing crosses involving linkage, it is more common to show them as vertical pairs, for example:*
- *This format will be used in examination papers, or students will be given sufficient information to allow them to deduce which alleles are linked.*

$$\frac{T\ B}{t\ b}$$

NATURE OF SCIENCE

Looking for patterns, trends and discrepancies: Mendel used observations of the natural world to find and explain patterns and trends. Since then, scientists have looked for discrepancies and asked questions based on further observations to show exceptions to the rules. For example, Morgan discovered non-Mendelian ratios in his experiments with *Drosophila*.

Dihybrid crosses

When we looked at monohybrid crosses, we took into account only one genetic trait. Sometimes it is interesting to study two at once. Let's consider Mendel's experiments with pea plants. In one cross, he examined the following two traits.

- Seed shape: some seeds are round, while others are wrinkled. The allele for round is dominant (see Figure 10.9);
- Seed colour: some seeds are green inside, while others are yellow. The allele for yellow is dominant (see Figure 10.9).

If a Punnett grid is set up for this cross and all the alleles could be shuffled in any random order, we find that there are 16 possible random combinations (see Figure 10.10). As we have seen before with **AA** and **Aa** genotypes in Section 3.4, some combinations of alleles can generate the same phenotype. If **R** represents round and **Y** represents yellow, both the genotype **RRYY** and **RrYy** would give seeds that were round and yellow. As long as R and Y are not linked genes, they should segregate independently. This means that they should be able to pass on to the next generation either with or without the other. They show no dependence on each other; no preference one way or the other. During the shuffling of alleles in meiosis, they are equally distributed between gametes. The result is that, in the offspring, there should be certain predictable ratios. These can be illustrated by Mendel's dihybrid cross, shown on the following page.

Phenotype = the resulting trait shown when a genotype is expressed, e.g. type O blood. Genotype = the two alleles of a gene received from the mother and father, e.g. AA, Aa, or aa.

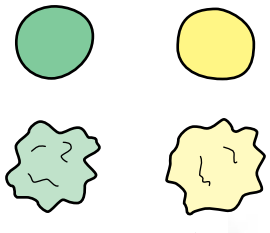


Figure 10.9 Seed colour and seed shape in peas.

Figure 10.10 A Punnett grid for inheritance of roundness and yellowness in pea seeds.

	RY	Ry	rY	ry
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRYy	RRyy	RrYy	Rryy
rY	RrYY	RrYy	rrYY	rrYy
ry	RrYy	Rryy	rrYy	rryy

Phenotypes

- = round yellow peas × 9
- = round green peas × 3
- = wrinkled yellow peas × 3
- = wrinkled green peas × 1

NATURE OF SCIENCE

Mendel crossed two true-breeding plants with each other. True-breeding means homozygous for the traits being studied; using them means no surprises can be produced by masked recessive alleles. One parent plant was homozygous dominant for both traits (round and yellow seeds), whereas the other parent was homozygous recessive for both traits (wrinkled and green).

To represent the alleles, Mendel used a system of letters that is incompatible with the system we use today so, in this example, Mendel's letters have been replaced with modern conventions:

- **R** = allele for round peas
- **r** = allele for wrinkled peas
- **Y** = allele for yellow peas
- **y** = allele for green peas.

Table 10.1 Phenotypes and genotypes of round/wrinkled and yellow/green peas

Parent phenotypes:	**round yellow**	**green wrinkled**
Parent genotypes:	**RRYY**	**rryy**
Parent gametes:	**RY**	**ry**
F_1 genotypes:	**RrYy**	
F_1 phenotypes:	**round yellow**	

F_1 refers to the first filial generation and it is the resulting offspring of the cross between the parents who are distinctly different (e.g. one green, the other yellow). F_2 refers to the second filial generation and they are the offspring of the F_1 generation.

The F_1 generation is made up exclusively of plants that have round yellow peas. When Mendel planted these peas, and let them grow into adult plants and self-pollinate, he expected some of the recessive traits to show up again in the F_2 generation. That did happen, and what is interesting is the ratio in which they appeared. From 15 plants, Mendel obtained 556 pea seeds in the following proportions:

- 315 round and yellow (56.6%)
- 101 wrinkled and yellow (18.2%)
- 108 round and green (19.4%)
- 32 wrinkled and green (5.8%).

If these percentages are converted to ratios, the numbers are close to the expected ratio for alleles that are passed on independently and are not found on the sex chromosomes. This ratio is 9:3:3:1 and is calculated using a 4 × 4 Punnett grid, as shown in Figure 10.10.

To set up the gametes for an F_2 Punnett grid, the FOIL method is employed: this is a mnemonic for 'first', 'outside', 'inside', and 'last' of the parents' genotypes, in this case **RrYy**. The first gametes take the first letter of each trait in the genotype: **RY**. The second gametes are formed using the outside letters of each trait **Ry**, then inside **rY**, and last **ry**.

The resulting ratio 9:3:3:1 indicates that, for every wrinkled green pea in the F_2 generation, there should be three round green peas. Mendel found 3.34 times more round green peas than wrinkled green peas in his experiment. There is often a difference between the theoretical values and actual values obtained in experiments. If thousands of seeds were examined instead of a few hundred, the number would probably be closer to three. A similar difference between theory and reality is seen in families where there are more or fewer boys than girls: few families have exactly 50% of each, despite the fact that the laws of genetics predict half and half. At the end of this section, we will use the chi-squared test to check whether Mendel's dihybrid results show any statistically significant difference between the expected values and the observed values.

CHALLENGE YOURSELF

2 Show the outcome of a cross between two homozygous parents: **AABB** × **aabb**.

An understanding of inheritance enables farmers to breed their livestock selectively for specific characteristics. Just as varieties of crops with the most desirable characteristics, such as highest yields (quantities of food grown), are selected for, and varieties with undesirable characteristics (such as susceptibility to frost, drought, or disease) are selected against, using artificial selection farmers select animals with the most desirable characteristics. For example, with the increase in popularity of mechanized milking machines to pump milk from cows instead of milking by hand, there has been an increased selection of cows with longer legs. If a cow has short legs, it is difficult to place the pumps on her udder, so such cows will not be selected for breeding. Likewise, varieties of cows that only produce 5–10 litres of milk a day will not be selected, whereas varieties that produce 30–40 litres of milk per day or more will be selected by breeders. Farmers today can choose the genetic makeup of their cows from a catalogue, and order gametes to be delivered in liquid nitrogen for the purpose of artificial insemination by a veterinarian. Thanks to such a system, as little as possible is left to chance, making sure that the farmer maximizes production.

Autosomes and sex chromosomes

As you saw in Section 3.2, in humans the sex chromosomes are the X and Y chromosomes, and they are the ones that determine whether a person is male or female. Any chromosome that is not a sex chromosome is called an autosome, or autosomal chromosome. Humans have 22 pairs of autosomes and one pair of sex chromosomes (see Figure 3.11 in Section 3.2).

If a trait or gene is described as autosomal, its locus is on one of the 22 pairs of autosomes, not on the sex chromosomes. A trait or gene that is said to be sex-linked must have its locus on a sex chromosome. Where a gene is located determines whether or not the trait it controls is more common in males or females. When a trait is more common in one sex than the other, the chances are that the trait is sex-linked, and that the locus of the gene is on either the X chromosome, the Y chromosome, or both (see Section 3.4). If there is no pattern in the frequency of a trait between females and males, it is probably an autosomal trait.

In his *Biographical Encyclopedia of Science and Technology*, Isaac Asimov points out how fortuitous it was that Mendel happened to choose traits of pea plants that were non-linked genes. The seven characteristics he studied were controlled by genes on separate chromosomes and therefore demonstrated independent assortment. Had he stumbled upon linked genes by bad luck, it is difficult to imagine how differently the history of the discovery of genetics would have played out. Would he have given up in frustration, or decided not to publish his discordant results? Or would he have figured out linked genes decades before anyone else discovered them?

Genetic disorders such as Huntington's disease and cystic fibrosis are autosomal disorders. Huntington's disease is caused by an autosomal dominant gene on the fourth chromosome, and cystic fibrosis is caused by an autosomal recessive gene on the seventh chromosome. Both disorders occur in similar proportions in males and females. Colour blindness or haemophilia, in contrast, are sex-linked disorders; their gene loci are both found on the X chromosome and both diseases affect males significantly more than females.

NATURE OF SCIENCE

Mendel's classic pea plant experiments clearly demonstrated that a monohybrid cross produces F_2 offspring in a 3:1 ratio, meaning that for every offspring showing a recessive phenotype, there are three offspring showing the dominant phenotype (see Figure 10.11).

	A	a
A	**AA**	**Aa**
a	**Aa**	**aa**

Three out of every four offspring show the dominant trait

One out of every four offspring shows the recessive trait

Figure 10.11 A Punnett grid showing a monohybrid cross.

Recall from Section 3.4 that a monohybrid cross is one that examines only one genetic trait, and its purpose is to see what kinds of offspring two parents with different alleles can produce.

In addition, as seen in Figure 10.10, a dihybrid cross gives a ratio of 9:3:3:1. Such ratios work reasonably well for many crosses. In the early 1900s, however, the pioneer geneticist Thomas Hunt Morgan made some observations that did not show such ratios. Instead of working with pea plants the way Mendel did, Morgan worked with the tiny fruit fly of the genus *Drosophila*.

Because fruit flies can produce a new generation every 2 weeks, Morgan was able to do thousands of crosses in his Fly Room at Columbia University, and discovered dozens of mutations never seen before. Among these mutations was one discovered in May 1910 for white eyes, easily detected amongst the red eyes of the normal (also called wild-type) flies. Some scientists might have ignored the white-eyed fly and excluded it from the experiments as an outlier, but Morgan and his team decided to see what would happen if it was allowed to breed.

A red-eyed wild-type fruit fly, and a white-eyed mutant fruit fly.

Because his team of researchers kept careful notes and statistics, some thought-provoking results started to emerge. When they crossed the white-eyed male with a red-eyed female, the results were as Mendel predicted (see Figure 10.12).

	W	0
W	WW	W0
w	Ww	w0

Three out of every four offspring show the dominant red-eyed trait

One out of every four offspring shows the recessive white-eyed trait

Figure 10.12 Crossing a white-eyed male with a red-eyed female fruit fly. Note that the letters and symbols that Thomas Hunt Morgan used a century ago are outdated and not used in modern Punnett grids.

W = allele for red eyes; **w** = allele for white eyes; **0** = absence of an allele (males have a Y chromosome that does not carry the gene for eye colour). Blue alleles shown are from the male parent, red from the female parent. Note that, today, we would use a different notation: sex-linked genes like this would be shown as $\mathbf{X^W}$, $\mathbf{X^w}$ or **Y**.

But look at what happened when Morgan's lab did the reciprocal cross, choosing a white-eyed female and a red-eyed male (see Figure 10.13).

	W	0	
w	Ww	w0	Two out of every four offspring show the dominant red-eyed trait
w	Ww	w0	Two out of every four offspring show the recessive white-eyed trait

The second cross does not follow Mendel's ratio of 3:1 and instead has a ratio of 1:1, because for every one fly that has red eyes, there is one that has white eyes. Clearly, something is different with genes found on the sex chromosomes (X and Y), and this is how Morgan discovered sex-linked genes. The chances of a characteristic showing up in one sex of fly is different from the chances of it showing up in the other because the eye-colour gene is found on *Drosophila*'s X chromosome. In the cross shown in Figure 10.13, all the males produced had white eyes and all the females had red eyes.

Morgan's team continued experimental crosses on thousands of fruit flies and thanks to their careful observations, diligent record keeping, insightful interpretations, and hard work, were able to provide concrete evidence for many of the things we learn about in genetics classes today: sex-linked genes, linked genes not found on the sex chromosomes, crossing over, gene mapping, and proof of the fundamental idea that chromosomes carry the genetic material that determines inheritance. For his contributions to the new science of genetics, Morgan won the Nobel Prize in 1933.

Figure 10.13 Crossing a white-eyed female and a red-eyed male fruit fly.

Thomas Hunt Morgan.

Linkage groups

As Morgan and his team demonstrated with their experiments on *Drosophila*, any two genes that are found on the same chromosome are said to be linked to each other (see Figure 10.14). Linked genes are usually passed on to the next generation together.

A group of genes inherited together because they are found on the same chromosome are considered to be members of a linkage group. This applies to genes found on autosomes as well as those on the sex chromosomes. In Figure 10.14, the green and yellow genes are linked. Neither is linked to the red genes.

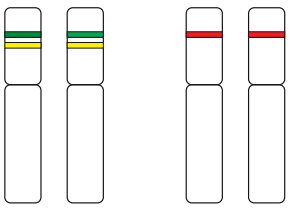


Figure 10.14 In these two pairs of chromosomes, can you see which genes are linked, and which are not?

Linked genes

In the fruit fly *Drosophila*, the gene for body colour (grey or black) is in the same linkage group as the gene for wing length (long or short) (see Figure 10.15). Again, this means that the loci for these two genes are located on the same chromosome and are therefore considered to be linked genes. The alleles are:

- **G** = allele for grey body
- **g** = allele for black body
- **L** = allele for long wings
- **l** = allele for short wings.

An example of linked genes in humans is haemophilia and colour blindness. Both genes are found on the X chromosome and, as a result, it is possible to inherit both together.

The genotypes of the true-breeding (homozygous) parents are:

- **GGLL** = genotype of a grey-bodied, long-winged parent
- **ggll** = genotype of a black-bodied, short-winged parent.

Fruit flies are bred in laboratories and used to study genetics. At the top of this tube it is possible to see adult flies and lower down (near the label) we can see them at earlier stages in their life cycle.

There is nothing in the genotype's notation **GGLL** that shows that **G** must be inherited with **L**. In order to show linkage, the following notation is used:

$$\frac{\mathbf{G}\quad\mathbf{L}}{\mathbf{G}\quad\mathbf{L}}$$

The two horizontal bars symbolize homologous chromosomes and show that the locus of **G** is on the same chromosome as **L**. One **G** is on the maternal homologue, and the other **G** is on the paternal homologue. Likewise, **ggll** is shown as:

$$\frac{\mathbf{g}\quad\mathbf{l}}{\mathbf{g}\quad\mathbf{l}}$$

To read the genotype of the individual for these two linked traits, the pairs of alleles are read vertically: the above symbol's genotype is **ggll**.

A cross between a homozygous dominant true-breeding fruit fly (**GGLL**) and a homozygous recessive true-breeding fruit fly (**ggll**) will result in flies that are all heterozygous for both of the traits (**GgLl**). The flies will all be grey with long wings, but they will all be carriers for the recessive alleles. Suppose such flies are accidentally put in the wrong jar in a laboratory along with another population of flies that look the same but that are all homozygous for both traits. Researchers in the lab would not be able to determine the genotype of any particular fly in the jar just by looking at it. A test cross with a known homozygous recessive would be necessary to determine whether a fly's phenotype is the result of a homozygous or heterozygous genotype (see Figure 10.16).

If the offspring of the test cross are all grey, long-winged flies, the unknown fly used in the cross must be homozygous (**GGLL**) for both traits. If the unknown fly has the heterozygous genotype **GgLl**, the resulting offspring will show some traits from each parent.

Figure 10.15 Test cross between a grey long-winged fly of unknown genotype with a black short-winged fly, a homozygous recessive.

Here are the linked genes in the heterozygote:

$$\frac{G\quad L}{g\quad l}$$

The test cross is done by mating an unknown fly (here heterozygous for both traits) with another that is known to be homozygous recessive for both traits:

$$\frac{g\quad l}{g\quad l}$$

One way of showing this cross is by drawing a Punnett grid (see Figure 10.16). A full 4 × 4 grid is not necessary because there are only four possible combinations:

	GL	Gl	gL	gl
gl	GgLl G L / g l	Gg ll G l / g l	ggLl g L / g l	gg ll g l / g l
		R	R	

Figure 10.16 Punnett grid showing the test cross. The two offspring labelled R are the recombinants (see text for explanation). Each box shows both ways of representing the genotypes.

Another way of showing the same idea is shown in Figure 10.17.

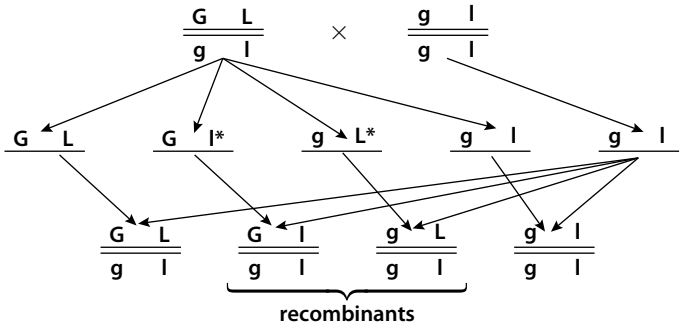


Figure 10.17 Text cross **GgLl** × **ggll**.

* produced by crossing over during meiosis

Look at the two possibilities for offspring in the middle of Figures 10.16 and 10.17 (the second and third possibilities in each case). By examining the alleles closely, it is possible to see that these offspring are different from either parent. A new shuffling of the alleles has created a new combination that does not match either of the parent's genotypes. The term recombinant is used to describe both the new chromosome and the resulting organism.

The way these recombinants form is through the process of crossing over. Without crossing over, the allele **G** would always be inherited with **L** for the simple reason that

they are linked. Thanks to crossing over, **G** sometimes gets inherited with **l**. In addition, **g** sometimes gets inherited with **L**, as seen in the recombinants in Figure 10.18.

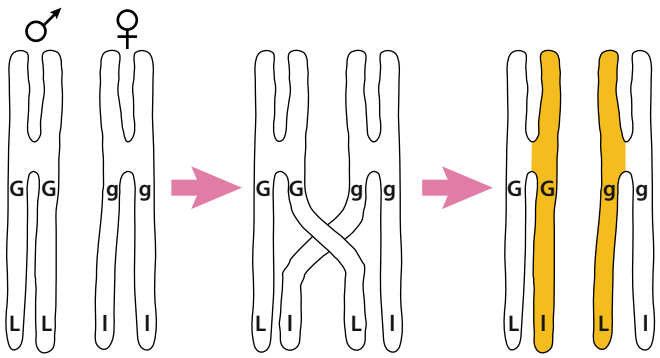


Figure 10.18 The highlighted chromatids show new combinations of alleles that were not observed in the original parents' chromosomes.

Outcomes of genetic crosses should typically follow Mendelian ratios of 3:1 for an F_2 monohybrid cross or 9:3:3:1 for an F_2 dihybrid cross. There is nothing alarming about a slight variation from these expected values, but if there is a significant deviation it suggests that independent assortment is not happening and, instead, the genes of the traits being observed are linked.

When gametes are made from the resulting bivalent shown on the right in Figure 10.18, two will contain combinations found in the parents (either **GL** or **gl**) whereas two will contain recombinants (**Gl** and **gL**). Thus, even in linked genes, nature has found a way to increase variety through crossing over.

Polygenic inheritance

Polygenic inheritance involves two or more genes influencing the expression of one trait. With two or more allelic pairs found at different loci, the number of possible genotypes is greatly increased. It is believed that most human traits are too complex and show too many combinations to be determined by one gene.

This could partly explain the difficulty in finding out which genes are responsible for traits whose genetic components are poorly understood, for example mathematical aptitude, musical talent, or susceptibility to certain illnesses.

Continuous and discrete variation

With dominant and recessive alleles of a single gene, the number of possible phenotypes is limited. For example, a person either has cystic fibrosis or not. When multiple alleles are introduced, the number of possibilities for a single trait increases accordingly. For example, the ABO blood type has three alleles and four possible phenotypes.

When a second gene is introduced, the number of possible genotypes increases dramatically. With three, four or five genes determining the phenotype, the number of possibilities is so big that it is impossible to see the difference between certain genotypes in the phenotype. When an array of possible phenotypes can be produced, it is called continuous variation. The colour of skin in humans is an example of continuous variation and it is thought that the intensity of pigment in skin is the result of the interaction of multiple genes.

In humans, continuous variation can also be seen in the genetic components of traits such as height, body shape, and intellectual aptitude. Each of these is also influenced by environmental components. A person's height, for example, is determined by whether he or she inherits genes for tallness, but it also depends on the person's nutrition as he or she is growing.

To help you decide whether or not a trait shows continuous variation, imagine a questionnaire to record phenotypes. In general, if it is possible to tick 'yes' or 'no' for a trait, that trait does not show continuous variation, for example dry versus wet earwax (see Section 3.1). The same is true for a trait whose possibilities can be represented by just a few choices, such as blood type (A, B, AB, or O).

When variation is not continuous, it is referred to as discontinuous variation. The data for discontinuous variation can be displayed as bar charts (see Figure 10.19). An unbroken transitional pattern from one group to another is not present.

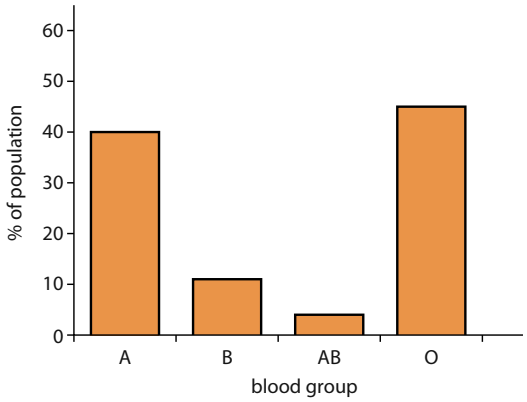


Figure 10.19 Blood type is an example of discontinuous variation.

Height in humans is an example of a trait that shows continuous variation.

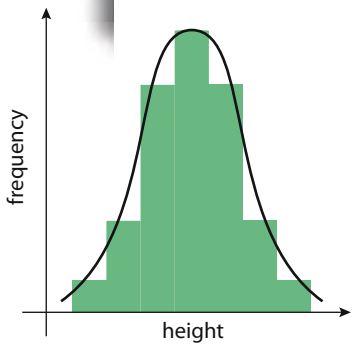


Figure 10.20 Height in humans is an example of continuous variation, with an even distribution around a mean.

Polygenic characteristics

Returning to the idea of a questionnaire about phenotypes, when there are many intermediate possibilities, then the trait shows continuous variation. If the results are plotted as a graph, it will produce a bell-shaped distribution curve. There is a smooth transition between the groups of frequencies (see Figure 10.20).

Is it fair to compare heights of humans from different parts of the world? On the one hand, we can argue that we are all the same species and therefore we are comparable. On the other hand, there has been a certain amount of isolation of gene pools over thousands of years, and we also know that different people grow up in very different environments. We are all the same. We are all different.

NATURE OF SCIENCE

Studies looking at identical twins, fraternal (non-identical) twins, and non-twin siblings have revealed that the heritability of height is about 80%. Heritability refers to how much of an effect genetics has on the trait. The other 20% of our height is the result of environmental factors. In human populations from different countries, people have different average heights. For example, as seen in the distribution curve in Figure 10.21, the average height for males is 1.83 m in the Netherlands, while it is 1.63 m in the Philippines.

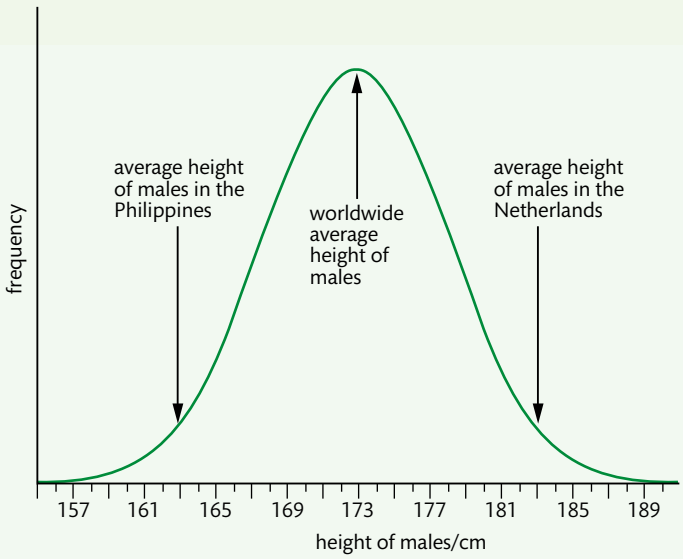


Figure 10.21 The worldwide average height of men.

Differences in height between one country's population and another could be explained in part by genetic factors, because people living on different continents have differing genetic backgrounds. However, within a region of the world where people have a similar recent genetic heritage, growth might be stunted by any number of environmental factors, such as:

- poor diet, notably a lack of protein, calcium, or vitamin D
- lack of exercise
- illness, which can sometimes be attributed to child neglect
- social problems, such as war, a low standard of living (poverty), or a low quality of life, which is often associated with substandard healthcare systems.

Groups that are interested in health issues, such as the World Health Organization, use height as one way of quantitatively measuring a population's health. An increase over time in the average height of children of a certain age indicates improvement in the health of a population, whereas stunted growth is an indicator of poor health in a population.

An example is the height differences seen in recent decades between North Koreans and South Koreans. Although the two countries share a common genetic heritage, the geopolitical split at the end of World War II has led to vast differences in the standards of living between them. A 2009 study published in the *Journal of Biosocial Science* found that pre-school children raised in North Korea are up to 13 cm shorter than children raised in South Korea. Famine and economic hardship in the 1990s contributed to widespread malnutrition in North Korea, leading to stunted growth, whereas South Korea has enjoyed economic expansion in recent decades and has seen a continued increase in height amongst its youth. Today, young men in South Korea are among the tallest in Asia.

This topic raises some interesting Theory of Knowledge questions.

TOK

In comparing heights between citizens of North Korea and South Korea, a crucial question arises: where do the statistics come from? How can we know if the official numbers released by the Democratic People's Republic of Korea are reliable? If the only other statistics for North Koreans come from measuring refugees fleeing the country, is this group a representative sample of the height of the country's population as a whole? Are the debates about height differences between North and South Korea simply being used as a political tool to criticize the policies of the Pyongyang regime? Or are they being used to support the argument that more food aid needs to be sent? Knowledge questions about limitations of information and bias of the sources of the information help us put such statistics into context and appreciate that a simple question such as 'How tall are they?' can have a complex and multi-faceted answer.

Chi-squared tests

When doing experiments about genetic characteristics being passed on from one generation to the next, it is expected, theoretically anyway, that certain ratios will be seen in the results. In practice, however, a sample size is far smaller than the whole population studied and, hence, the ratios found in experiments are rarely equal to the expected ratios. This can be seen in Mendel's experiments with pea plants (see Figure 10.22).

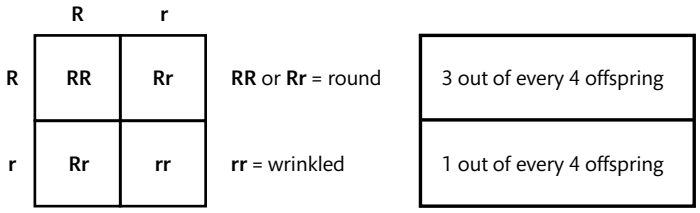


Figure 10.22 Mendel's pea experiment.

In this monohybrid cross looking at round peas versus wrinkled peas, Mendel expected a ratio of 3:1. Instead, when he counted up 7324 seeds from his experiment, he got a ratio of 2.96:1. The question is: does that pose a problem? Could this mean that something is wrong, or that another factor besides independent assortment is affecting his results? Could there be some other mechanism that would favour one allele over another and generate results that deviate from the expected ratio?

One statistical test that biologists use to see whether the difference between an observed result and an expected result is statistically significant is the chi-squared (χ^2) test (the Greek letter chi, χ, is pronounced like 'sky' without the s). The chi-squared test helps us to see statistically whether or not there is a good fit between a theoretical model (in this case the expected ratios in a Punnett grid) and what really happens in nature. The worked example below is an application of the χ^2 test on Mendel's results.

Worked example

Use the χ^2 test on Mendel's monohybrid cross of round and wrinkled pea seeds to determine whether there is a statistically significant difference between the expected ratios and the observed ratios.

Solution

To use this statistical test, it is important to record carefully all the observed results (O) and the expected results (E). The expected results are what can be calculated theoretically and, in genetics exercises, this means using a Punnett grid to determine ratios of offspring. Because 7324 seeds in total were collected and we expect 3 out of every 4 offspring to be round, then the expected value for round seeds is 5493, as shown in Table 10.2.

Table 10.2 Observed and expected ratios of round and wrinkled pea seeds

	Round	Wrinkled	Total
Observed phenotypes (O)	5474	1850	7324
Expected proportions	3 out of every 4 offspring	1 out of every 4 offspring	
Expected phenotypes (E)	3 out of every 4 offspring from a total of 7324 = 5493	1 out of every 4 offspring from a total of 7324 = 1831	7324
Difference (O – E)	–19	19	
Difference squared $(O - E)^2$	361	361	
$\frac{(O - E)^2}{E}$	0.066	0.197	0.264

The fourth and fifth white rows of this table are intermediate steps to find the difference between the observed and the expected values, and their squared values. Note that the values are squared in order to be sure that the negative sign does not pose a problem.

The bottom right cell of the table is what is needed: it shows the sum of the last row's values and this is the χ^2 value we are interested in. The contents of Table 10.2 can be summarized in the generalized formula for calculating χ^2, which is:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

χ = Greek letter chi

O = observed values (the results of the experiment)

E = expected values (calculated theoretically)

$\sum$ = sum of all the calculations for each type of outcome

Note that the chi-squared test should only be used if the size of the sample observed is greater than 30. Ideally, 50 or more observed values would be better. As Mendel's experiment in this example had thousands of results, we can use this test with confidence. Also note that all of the expected values should be equal to or greater than 5. Again, with Mendel's experiment (5493 round peas expected and 1831 wrinkled peas expected), we can use the chi-squared test with confidence that it will give a reliable result.

Interpreting the χ^2 value calculated

Now that we know the χ^2 value for Mendel's experiment, we need to know what it means. For this there are some concepts that need to be clarified. First of all, there is the concept of the null hypothesis (H_0). The H_0 in an experiment of this type is what we would expect: in Mendel's example, we expect a 3:1 ratio. The χ^2 value will help us determine whether the null hypothesis can be accepted or rejected. Accepting the null hypothesis is a way of saying 'Yes, the alleles are passed on in a random fashion and there is a high probability that any deviation from the expected values can be attributed to chance'. In other words, we have a high level of goodness-of-fit between the theoretical model and the values obtained, and we do not need to worry about the deviation from the expected values, which might be because of unseen and unexpected factors.

Another two important concepts to understand are the degrees of freedom (d.f.) and how the idea of probability (p) is used. The degree of freedom is determined by taking the number of classes into which the data fall and subtracting 1 from that number. In the case of round versus wrinkled seeds, there are two classes into which

the data fall, so there is 2 – 1 = 1 degree of freedom. This number lets us to know where to look in a table of critical values for χ^2 (Table 10.3). Notice in Table 10.3 that, in addition to the degrees of freedom, there are probability values for *p*. It is a convention in biology to look for probabilities of 5%, or 0.05.

Table 10.3 Critical values for χ^2

		Probability values (*p*)				
		0.1	**0.05**	**0.025**	**0.01**	**0.005**
Degrees of freedom (d.f.)	**1**	2.706	3.841	5.024	6.635	7.879
	2	4.605	5.991	7.378	9.21	10.597
	3	6.251	7.815	9.348	11.345	12.838
	4	7.779	9.488	11.143	13.277	14.86
	5	9.236	11.07	12.833	15.086	16.75
	6	10.645	12.592	14.449	16.812	18.548
	7	12.017	14.067	16.013	18.475	20.278
	8	13.362	15.507	17.535	20.09	21.955
	9	14.684	16.919	19.023	21.666	23.589
	10	15.987	18.307	20.483	23.209	25.188

Look at Table 10.3 and find the critical value that is of interest to us: it is the one that lines up with a probability value of 0.05 and a degree of freedom of 1. You should get 3.841. This means that any value we calculate for χ^2 that is greater than 3.841 tells us to reject the null hypothesis. Conversely, if we end up with a calculated value that is less than 3.841, it means we can accept the null hypothesis. In the present case, our calculated value is 0.264, which is less than 3.841, so we can accept the null hypothesis. This information can be interpreted as:

- there is at least a 95% probability that the deviation of 19 seeds from the expected values can be attributed to chance
- there is at most a 5% chance that there might be another factor influencing the results.

The worked example illustrates how you need to follow a certain number of steps so that the χ^2 test can help you determine whether your results are statistically significant, or whether something unexpected is altering the results more than would be expected by chance. Here is a summary of the steps.

- Determine the expected values (E) (although we sometimes like to use percentages or proportions in science, the chi-squared test requires actual numbers, so do not use percentages or ratios).
- Note down the observed values (O) and decide what the null hypothesis will be (in genetics problems, the null hypothesis usually states that the ratios should be the ones calculated in Punnett grids).
- Calculate the value for χ^2 by determining the differences between the values (O – E), then square them $(O-E)^2$, and finally divide by E and add up all the values you got for $\frac{(O-E)^2}{E}$. This sum is the χ^2 value.

You need to become comfortable with using the steps of the chi-squared test. You do not need to memorize the tables for *p* values. However, it is recommended that you practise with several different problems so that you understand how it works.

- Determine the degrees of freedom (d.f.) by taking the total number of classes into which the data fall and subtracting 1.
- Look at the table of critical values of χ^2 and use the d.f. and *p* value (conventionally we use 0.05 for *p*) to determine which critical value ($\chi^2_{critical}$)to compare the calculated value of χ^2 ($\chi^2_{calculated}$) to.
- Compare $\chi^2_{critical}$ with $\chi^2_{calculated}$ and decide if the null hypothesis can be rejected or accepted using the rules shown in Figure 10.23:

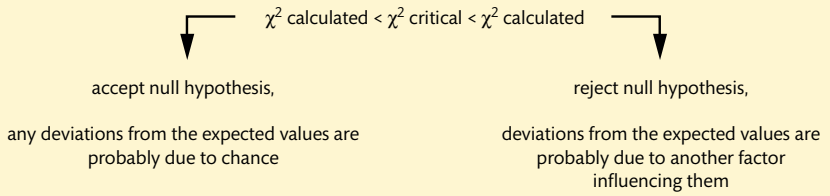


Figure 10.23 The rules for applying χ^2 values.

If the calculated value for chi-squared is less than the critical value, the null hypothesis can be accepted. If the calculated value for chi-squared is greater than the critical value, the null hypothesis can be rejected.

CHALLENGE YOURSELF

3 Using the information given at the beginning of Section 10.2, about Mendel's experiment on the traits in peas of seed colour and seed shape, calculate the chi-squared value and determine whether or not there is a significant difference between the observed and expected values. To help you, the following table (Table 10.4) has been set up and the first row completed. To answer the question, you will need to recall that we expect a 9:3:3:1 ratio, meaning that from the total of 556 peas, 9 out of every 16 are expected to be round and yellow, 3 out of every 16 are expected to be round and green, etc.

Table 10.4 Mendel's dihybrid cross

	Round yellow	Round green	Wrinkled yellow	Wrinkled green	Total
Observed (O)	315	108	101	32	556
Expected (E)					
Difference squared $(O-E)^2$					
$\frac{(O-E)^2}{E}$					

Once you have worked out the value for the bottom right cell of the table, you have the chi-squared value that must be compared with the critical values table (Table 10.3). Remember to determine the degrees of freedom and to use 0.05 as the value for *p*.

4 William Bateson and Reginald Punnett (the man whose grids we use to calculate expected offspring) continued Mendel's work on pea plants, and tried other traits besides the seven that Mendel looked at. Table 10.5 shows the results for an experiment using purple flowers/red flower as one trait and long pollen grains/round pollen grains for the second trait in their dihybrid cross (in this plant, purple and long are the dominant traits). The parents who produced this F_2 generation were **PpLl**.

Table 10.5 Linked genes

	Purple long	Purple round	Red long	Red round	Total
Observed	284	21	21	55	381

Using the information about the observed values, set up a similar table and do the same calculations as for question 3. Explain why the results of this cross do not show the expected 9:3:3:1 ratio. Identify the offspring that represent recombinants.

Exercises

4 Define the term linkage group.

5 A genetic disease can be described as being an autosomal dominant disease. From this terminology, what can be deduced about the locus of the gene that causes such a disease?

6 The parents in a cross are **AaBb** and **aabb**, respectively.

(a) Draw diagrams for each showing that the two different genes are linked.

(b) Show how the offspring in the cross are produced and clearly label the recombinants.

To learn more about Thomas Hunt Morgan and his work, along with more information about genetics, and about chi-squared tests, go to the hotlinks site, search for the title or ISBN, and click on Chapter 10: Section 10.2.

10.3 Gene pools and speciation

Understandings:

- A gene pool consists of all the genes and their different alleles, present in an interbreeding population.
- Evolution requires that allele frequencies change with time in populations.
- Reproductive isolation of populations can be temporal, behavioural, or geographic.
- Speciation due to divergence of isolated populations can be gradual.
- Speciation can occur abruptly.

Applications and skills:

- Application: Identifying examples of directional, stabilizing, and disruptive selection.
- Application: Speciation in the genus *Allium* by polyploidy.
- Skill: Comparison of allele frequencies of geographically isolated populations.

Guidance

- *Punctuated equilibrium implies long periods without appreciable change and short periods of rapid evolution.*

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Looking for patterns, trends, and discrepancies: patterns of chromosome number in some genera can be explained by speciation due to polyploidy.

Interbreeding populations

The gene pool is all the genetic information present in the reproducing members of a population at a given time. The gene pool can be thought of as a reservoir of genes from which the population can get all its various traits. A large gene pool exists in a population that shows substantial variety in its traits, whereas a small gene pool exists in a population whose members show little variation, notably in cases of inbreeding. Inbreeding is the practice of having closely related organisms mate with each other.

Allele frequency is a measure of the proportion of a specific variation of a gene in a population. The allele frequency is expressed as a proportion or a percentage. For example, it is possible that a certain allele is present in 25% of the chromosomes studied in a population. This would mean that one-quarter of the loci for that gene are occupied by that allele, and the other three-quarters do not possess it.

This could also be interpreted as a 25% chance that a chromosome in that population has the allele at that particular locus. Note that this does not mean that 25% of the members of the population have the allele. Later in this section we will see how these numbers play out in a diploid situation where two chromosomes in each organism can carry a version of the gene.

Look at Figure 10.24. The gene pool in this population of 16 people is made up of 32 genes. Count the number of **T**s. You should get 16. Do you get the same for the number of **t**s? You should. Because half are **T**s and half are **t**s, the allele frequency for

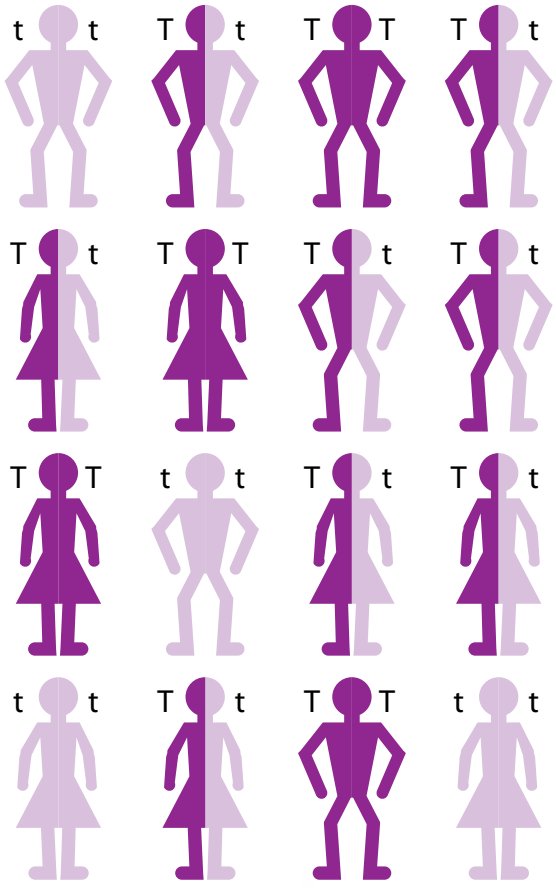

Figure 10.24 In this gene pool, the frequencies of each allele **T** and **t** is 50%.

each is 50%, or 0.50. Does this mean that half the people have the phenotype caused by the recessive allele? No. Only four people are homozygous recessive and have the phenotype, which is 25% of the population. Be careful not to confuse allele frequency with the number of people who show a particular trait.

Evolution and allele frequencies

Gene pools are generally relatively stable over time. But not always. New alleles can be introduced as a result of mutation, and old alleles can disappear when the last organism carrying the allele dies. One result of evolution is that, after many generations of natural selection, some alleles prove to be advantageous and tend to be more frequent.

Conversely, some alleles are disadvantageous to the survival of the organisms in the population, and are not passed on to as many offspring. From this it should be clear that, at any time an allele frequency is estimated, it is only a snapshot of the alleles at that time. Several generations later, the proportions of alleles may not be the same.

In addition, if populations mix as a result of immigrations, there will probably be a change in allele frequencies. The same is true for emigrations, when one group with a particular allele leaves the population. Whatever the reason, if a gene pool is modified and the allele frequencies change, we know that some degree of evolution has happened. No change in allele frequencies, however, means no evolution.

The Hardy–Weinberg equation

In order to calculate the frequencies of alleles, genotypes, or phenotypes within a population, the Hardy–Weinberg equation is used. This equation is useful for determining how fast a population is changing, or predicting the outcomes of matings or crosses. To understand how it is used, it is best to start with understanding how it was derived.

You will recall from Section 3.4 that a Punnett grid shows the genotypes of the parents and offspring in a cross. For the Hardy–Weinberg equation, we need to look at the cross in a new way: as a model for the allele frequencies. To do this, we need the variables p and q:

- p = frequency of the dominant allele (allele **T** in the example below)
- q = frequency of the recessive allele (allele **t** in the example below).

When looked at individually, the frequencies of the alleles on chromosomes must add up to 1. So $p + q = 1$. For example, if $p = 0.25$ (or 25%) frequency, then $q = 0.75$ because whichever chromosomes do not have the dominant allele must carry the recessive one.

What complicates things is the fact that we usually want to consider diploid organisms that carry two copies of any particular gene. As a result, the equation becomes $(p + q)^2 = 1$. If you remember your mathematics classes about polynomials, you'll know that $(p + q)^2$ can be expanded to $p^2 + 2pq + q^2$.

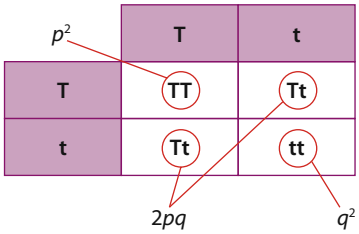

Figure 10.25 Annotated Punnett grid showing allele frequencies.

We can now deduce that $p^2 + 2pq + q^2 = 1$. This mathematical representation for the allele frequencies is known as the Hardy–Weinberg equilibrium and it is reached after only one generation of random interbreeding.

If this is still a bit confusing, try looking at it this way. Let's summarize the Punnett grid in Figure 10.25 as shown in Table 10.6.

Table 10.6 The Hardy–Weinberg equilibrium

	One square	Two squares	One square
Genotypes	**TT**	2**Tt**	**tt**
Proportions	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{4}$

Looking again at the Punnett grid in Figure 10.25, in terms of the allele frequencies rather than genotypes, the following can be deduced:

- the frequency of **TT** = p^2
- the frequency of **Tt** = $2pq$
- the frequency of **tt** = q^2.

By adding up all the possible proportions, we can see that $\frac{1}{4} + \frac{1}{2} + \frac{1}{4}$ comes to a total of 1. Based on the frequencies, we can replace the proportions, as shown in Figure 10.26.

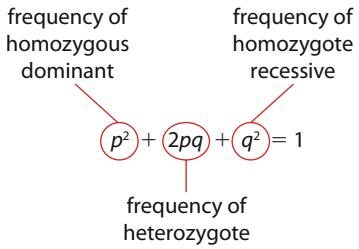

Figure 10.26 Annotated Hardy–Weinberg equation.

Before you move on to the next section, try the equation. If you substitute 0.25 for p and 0.75 for q in the equation, you should get 1. Be careful with the order of operations: anything that needs squaring should be done first, then perform any multiplications, then the additions.

Note that this equation gives mathematical support to the idea in Mendelian genetics that variation must be preserved from generation to generation. It is one of the characteristics of genetics that allows a population to be successful.

Here is a series of worked examples to help you get some hands-on experience: you will not be able to understand fully how to use the Hardy–Weinberg equation without practice.

Worked example

Calculating allele frequency, part I

Let's consider a disease caused by a recessive allele **t**. Let's say that the predicted frequency of this allele in the population being studied is 10%. Calculate the frequency of the healthy allele in the population.

Solution

From the information given, we know that q is 0.10, and as the proportions of p and q must always add up to 1, we can say that $p = 1 - q$. So p must be 0.90, which means that, in the gene pool, 90% of the alleles are **T**.

Remember that this does not mean that 90% of the population is healthy, because we are calculating an allele frequency, not a genotype frequency.

Calculating allele frequency, part II

In a study of 989 members of the population from example 1, it was found that 11 people had the disease. Calculate the frequency of the recessive allele **t**.

Solution

First, calculate the proportion of people who had the disease (which in this case is the proportion of **tt** genotypes). To do this, divide 11 by 989 to obtain 0.011. This means that 1.1% of the population has the genotype **tt**. Hence, $q^2 = 0.011$.

So to calculate q, we take the square root of 0.011, which gives us 0.105. This means that the frequency of the recessive allele **t** is 10.5% in this population. Note that this is very close to the predicted value of 10% in example 1.

Calculating genotype frequency

Using the information from example 1, consider the following.

(a) Fill out a copy of Table 10.7.

Table 10.7

Allele frequencies	Recessive **t**	q	
	Dominant **T**	p	
Genotype frequencies	Homozygous recessive **tt**	q^2	
	Heterozygous **Tt**	$2pq$	
	Homozygous dominant **TT**	p^2	

(b) Calculate the frequency of carriers in 500 members of the population.

Solution

(a) We know from the data given in part I of the worked example that $q = 0.10$ and that $p = 0.90$. These values will complete the first two rows of the last column of the table.

To fill in the other cells, simply perform the mathematical operations in the third column.

$q^2 = 0.01$, so 1% of the population is tt.

$2pq = 0.18$, so 18% of the population is Tt.

Lastly $p^2 = 0.81$, so 81% of the population is TT.

(b) To find the number of carriers (heterozygotes) in 500 members of this population, multiply 500 by 18% to get 90. So 90 people would be carriers.

Calculating phenotype frequency

Using information from Table 10.7, calculate the number of people amongst 500 members of the population who do not have the disease.

Solution

Using the numbers calculated in the previous worked example, we can complete the table as shown in Table 10.8.

Table 10.8

Allele frequencies	Recessive **t**	q	0.10
	Dominant **T**	p	0.90
Genotype frequencies	Homozygous recessive **tt**	q^2	0.01
	Heterozygous **Tt**	$2pq$	0.18
	Homozygous dominant **TT**	p^2	0.81

We know that, in order to not have this disease, a person must be either **TT** or **Tt**. The combined percentages of these genotypes is 81% + 18%, which gives 99%. 99% × 500 = 495. So 495 people out of the 500 should have the healthy phenotype.

Reproductive isolation of populations

In some situations, populations of members of the same species (and thus of the same gene pool) can be stopped from reproducing together because there is an insurmountable barrier between them. Such a barrier can be geographical, temporal, behavioural, or related to the infertility caused by hybridization. Each one of these will be explored below.

Geographical isolation

Geographical isolation happens when physical barriers, such as land or water formations, prevent males and females from finding each other, thus making interbreeding impossible. For example, a river, a mountain, or a clearing in a forest could separate populations. Tree snails in Hawaii demonstrate this geographical isolation: one population lives on one side of a volcano and another population lives on the other side, and they never come into contact with each other.

A waterway or mountain range can physically separate two populations, causing geographical isolation.

Temporal isolation

Temporal isolation refers to incompatible time frames that prevent populations or their gametes from encountering each other. For example, if the female parts of the flowers of one population of plants reach maturity at a different time compared with the release of pollen of another population, the two will have great difficulty producing offspring together. Or if one population of mammals is still hibernating or has not returned from a migration when another population of the same species is ready to mate, this would also be a temporal barrier between the two gene pools.

Behavioural isolation

Behavioural isolation can happen when one population's lifestyle and habits are not compatible with those of another population. For example, many species of birds rely on a courtship display in order for one sex to copulate with the other. If the males of one population has a version of a courtship display that is significantly different from another population, the females may not find the males of the other population seductive enough to be potential mates. Hence little or no reproduction will take place between the members of the two populations because of behavioural differences.

Directional, stabilizing, and disruptive selection

When a phenotype is favoured over another by natural selection, it is called directional selection. In such a case, the frequency of one phenotype is seen to increase over time, whereas the other phenotype decreases. This can occur when an organism's environment changes. We have seen this with industrial melanism (Section 5.1), when the lighter-coloured peppered moth's, *Biston betularia*, frequency in the population decreased during the industrial revolution, while the darker phenotype increased in the population. Another way of thinking about directional selection is to think of it as selection away from one extreme.

When one phenotype is favoured over two extreme phenotypes, it is called stabilizing selection. For example, a flowering plant might make some flowers with more nectar and some flowers with less nectar, but because producing excessive nectar would be a drain on the plant's sugar resources, and producing too small a quantity would discourage insects from returning, an intermediate quantity is produced that is a balance between too much and too little. Another way of thinking about stabilizing selection is to think of it as selection away from two extremes or selection towards the mean.

When two extreme phenotypes are favoured by natural selection, rather than one intermediate phenotype , it is called disruptive selection. Sometimes it is an advantage to have two opposing varieties of a phenotype rather than only one. For example, tadpoles of spadefoot toads in the Americas have two possible morphologies, one for an omnivorous diet (in *Spea muliplicata*) and one for a strictly carnivorous diet (in *Spea bombifrons*) that includes cannibalism if food sources are scarce. Having two separate morphologies gives these species a better chance of survival in places where water supply and food sources are variable. Another way of thinking about disruptive selection is to think of it as selection against the mean. The idea is to maintain two different phenotypes within the population. If the differences caused by disruptive selection are extreme and the two populations occupy different niches, it is possible for speciation to occur.

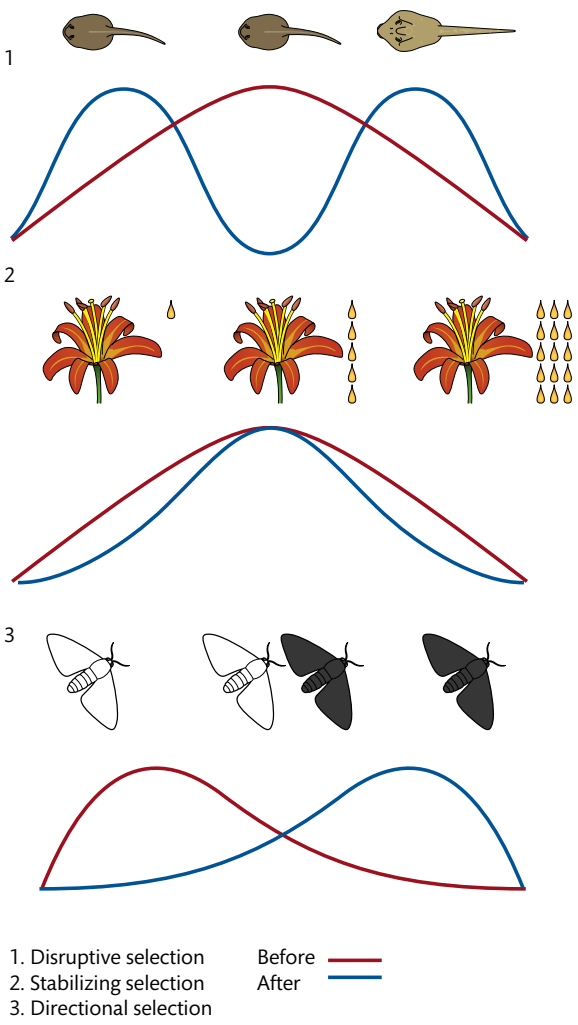

Figure 10.27 Three different types of disruptive selection: (1) two types of spadefoot tadpoles (omnivore or carnivore); (2) the amount of nectar in lilies; and (3) the frequency of the two forms of the peppered moth.

A blackcap

CHALLENGE YOURSELF

5 Determine whether each of these examples is of directional, stabilizing, or disruptive selection.

(a) A species of tropical land snail, *Amphidromus martensi*, shows a polymorphism of opposite-coiling shells. Some snails in the species have shells that coil in a clockwise manner and others in an anti-clockwise manner.

(b) Human babies demonstrate different body masses at birth, but a body mass that is too small would be disadvantageous to the survival of the baby. Likewise, a body mass that is too great would make childbirth difficult and could also reduce the baby's chances of survival. As a result, human body mass at birth tends to stay within reasonable limits of the mean.

(c) A species of bird is used to laying its light-coloured eggs on the white pebbles of a beach, and has done so for many generations. The light colour helps to hide the eggs from predators and it is extremely rare to see any dark eggs produced. Because of a volcanic eruption a decade ago that covered the beach in black particles, this particular species of bird is now laying more and more dark-coloured eggs.

(d) A species of bird called the blackcap lives in Germany and generally migrates to south-western parts of Europe for the winter to find food. Migration is an innate behaviour and is therefore an expression of genetic information. In recent decades, a mutation has occurred that causes some birds in the population to migrate to the west towards the UK. Because many people put out bird seed in their gardens all winter, the blackcaps in the UK population are surviving. There are now two separate migratory patterns in this species of bird.

(e) Giraffes used to have shorter necks. As time went on, extremely long necks were selected for and short or medium-length necks were selected against.

6 When a person's blood type is O^+, the positive sign refers to a genetic characteristic called the Rhesus factor, Rh. **D** denotes the allele for Rh^+ and **d** denotes Rh^-. Anyone with the genotype **dd** has a Rhesus negative (Rh^-) phenotype and anyone with the genotypes **DD** or **Dd** has the Rhesus positive (Rh^+) phenotype. Below are some results from two studies about the allele frequency of this gene in two different parts of the world.

- In Lagos, Nigeria, a study of 23 832 people revealed that 3% of the population was Rh^-.
- In Abha, Saudi Arabia, a study of 944 males revealed that 7.2% of the population was Rh^-.

(a) Using this information and applying the Hardy–Weinberg equation, calculate the frequencies of the alleles **D** and **d** in each of the two countries.

(b) In the south-west of France, a study of 127 French Basques found an allele frequency for **d** to be 0.51. How does this compare with the frequency of **d** in the two other populations?

Polyploidy

You will recall that haploid cells, such as sex cells, contain one set of chromosomes (n). This can be referred to as monoploidy. Diploid cells, such as somatic cells, contain two sets of chromosomes ($2n$): one from each parent. Polyploidy refers to the situation in which a cell contains three or more sets of chromosomes ($3n$, $4n$, and so on):

- $3n$ = triploid
- $4n$ = tetraploid
- $5n$ = pentaploid.

Such a situation can arise when cell division does not completely separate the copies of chromosomes into distinct nuclei, and they end up in the same cell. Polyploidy is much more common in plants than in animals. In plants, the extra sets of chromosomes can lead to more vigorous plants that produce bigger fruits or food storage organs and are more resistant to disease.

Having extra sets of chromosomes has the consequence of making errors in replication more common. If one population of plants is triploid and another tetraploid, each population's evolution will be different. If one population evolves at a different rate from another, the two could become so dissimilar that they no longer belong to the same species.

The process of an evolving population changing significantly enough so that production of offspring with the original population becomes impossible is called speciation. In short, a new species has evolved from the old one and both will continue in their separate ways (see Figure 10.28).

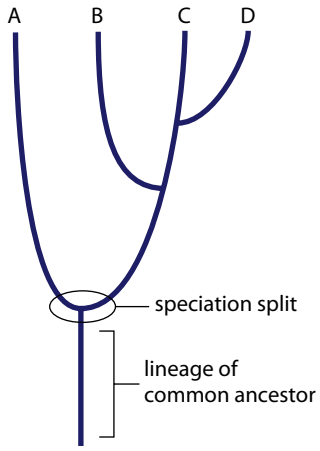

Figure 10.28 Species A, B, C, and D evolved from a common ancestor. There were three speciation splits to generate these species, the first of which is circled.

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Certain plants show interesting chromosome combinations that suggest that there were doublings of the chromosome numbers and hybridizations (recall from Chapter 3 that a hybrid can be formed by mating two individuals of different species). For example, the genus *Allium*, which includes plants you probably know well, such as onions, shallots, leeks, and garlic, as well as some popular ornamental flowers, shows polyploidy.

Botanists studying the chromosomes of the *Allium* genus noticed that the vast majority of species have a chromosome number of $x = 8$, although sometimes species can have $x = 7$ or $x = 9$. The letter x represents what is called the basic number of chromosomes. It is the largest common factor in a series of related species showing polyploidy – any chromosome number from any of the related species should be divisible by x. Most frequently, when $x = 8$, plants are diploid ($2n = 2x = 16$) but some species can have other multiples of 8: triploid ($2n = 3x = 24$), with three sets of chromosomes, or tetraploid ($2n = 4x = 32$), with four sets of chromosomes.

The most commonly cultivated species of onion is *Allium cepa*, but this species can have many varieties and the varieties can have strains. For example, the vast majority of strains of the *viviparum* variety of *A. cepa*, known as the Egyptian walking onion, is diploid. However, there is a strain called Ljutika, grown in Croatia along the coast of the Adriatic Sea, that has been found to be triploid.

The Egyptian walking onion, *Allium cepa* var. *viviparum*.

Analysis of various karyotypes has revealed that the Egyptian walking onion, *A. cepa* var. *viviparum*, is a hybrid between two *Allium* species: *A. fistulosum* and *A. cepa*. Because of its hybrid nature, it is sterile, so gardeners and farmers who want to grow this plant allow it to propagate vegetatively, which it can do because the bulbs can split and multiply underground from one year to the next. Also, the onion heads that you see in the photo can touch the ground when the stalk bends, and this will generate a new plant a few centimetres away from the original plant. Imagining how this progresses from year to year makes it easy to guess how this plant got its name.

The subgenus *Microscordum* contains species that are tetraploids, meaning $2n = 4x = 32$ (a total of four sets of eight chromosomes). The fact that similar related species show multiples of eight chromosomes suggests speciation by polyploidy.

Although polyploidy is rare in animals, certain species of the whiptail lizard in the genus *Aspidoscelis* show polyploidy and can reproduce without a male. The eggs can develop into baby lizards without being fertilized. This is a distinct advantage for colonizing new territories, as only one female is needed to produce a population. All the descendants are female lizards. This kind of reproduction is called parthenogenesis.

Recall that a karyotype is a property of a cell that is determined by the number and shapes of chromosomes that are present.

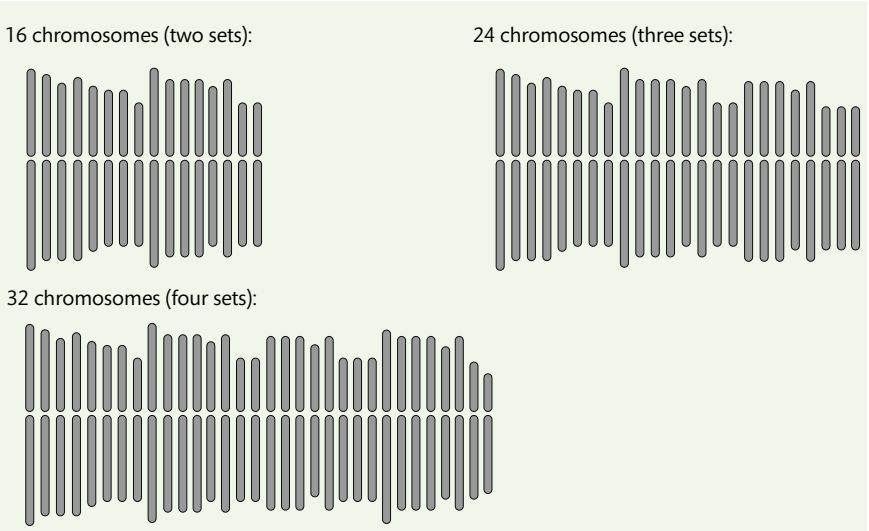

Figure 10.29 In a plant species, when the basic number of chromosomes (x) is 8, that means diploid cells (2x) have 16 chromosomes, triploid cells (3x) have 24, and tetraploid (4x) have 32 chromosomes.

When choosing which crops to plant, modern farmers tend to look for the plants that will have the best growth and produce the most food per hectare (best yield), because their income is based on production. Plant hybrids can often lead to just such qualities, including bigger grains, fruits, or vegetables, as well as hardier plants that can respond more favourably to the application of fertilizer. Think about the opposite of hybridization: inbreeding. With inbreeding, the genetic variety is reduced and the risk for disease increases, with a lack of vigour. Hybrids, on the other hand, such as most of the varieties of corn grown in the world, have increased yields.

Many of the food plants we eat today are hybrids, including corn, *Zea mays*.

Speciation due to divergence of isolated populations

Among evolutionary biologists there is some discussion as to the rate at which species evolve. Generally it is agreed that evolution does not happen overnight, but there are two main views (see Figure 10.30):

- the changes are small, continuous and slow (gradualism)
- the changes are relatively quick and followed by long periods of little or no change (punctuated equilibrium).

Gradualism was first proposed in the late 18th century in reference to geological ideas. It was adopted by Charles Darwin in relation to evolution. Supporters of this view argue that the fossil record shows a succession of small changes in the phenotypes of species, indicating that the process of speciation is a steady, ongoing one, with transitional stages between major changes in a phylogenic line. In addition, they

argue that, because we do not see rapid evolution happening today in nature, we can conclude that the process happens gradually.

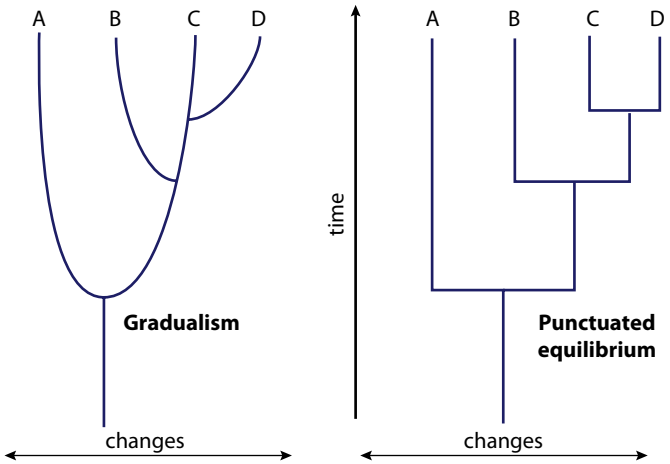

Figure 10.30 Gradualism and punctuated equilibrium are two contrasting views concerning the rate of speciation and evolution.

In contrast, those who support punctuated equilibrium, a theory that originated in the late 20th century, argue that speciation happens quickly, often in response to a change in the environment, for example after a volcanic eruption, a meteorite impact, or a major climate change. In response, some species are destroyed and others adapt to their new surroundings, exploiting the niches made available by the extinction of competing species. This was certainly the case for the species of mammals that took over the habitats abandoned by dinosaurs 65 million years ago.

In the absence of speciation, species can live for millions of years with little or no change. This has been confirmed in the fossil record for some species, notably sharks, cockroaches, and horseshoe crabs, which have persisted for hundreds of millions of years.

Critics of punctuated equilibrium argue that the 'jumpy' effect of this theory could simply be an artefact of the incompleteness of the fossil record. Discontinuities in the fossil lineages are a challenge for scientists to explain.

One difficulty of supporting either claim is that the only evidence we can use is from fossils. Many of the things that help to define a species are preserved poorly in fossils or not preserved at all, such as pigmentation, behaviour, and mating calls and songs.

Another argument is that, just because a fossil of an extinct crocodile looks very similar to a modern-day crocodile, there is no proof that the latter is a direct descendent of the former or that the two species would have been able to reproduce together had they been contemporaries.

The underside of a horseshoe crab (*Limulus polyphemus*).

In science, theories are like pairs of glasses. If you adhere to a certain theory, observations you make will be interpreted with the objectives of the theory running through your head. If you are a supporter of the gradualism theory, as Darwin was, you will tend to see the fossil record through that pair of glasses. If you take off those glasses and try on a different pair, in other words if you embrace a different theory, you might see the same scientific evidence in a totally different light. This change in view is called a paradigm shift, and such shifts in thinking happen regularly in science when new theories are developed. What factors contribute to the success of a paradigm and what factors might cause a paradigm shift?

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Richard Dawkin's book *The Greatest Show on Earth* provides some examples of modern-day rapid change in a species. One is a thought-provoking experiment started in 1971 on an island in the Adriatic Sea called Pod Mrcaru, off the coast of Croatia. Five pairs of the Italian wall lizard, *Podarcis sicula*, were introduced to the small island; when researchers returned in 2004 they were astounded to find the island covered with them. Genetic fingerprinting techniques confirmed that all the subjects tested were descendants of the original introduced lizards, but they were very different from their ancestors. Whereas the original species had had a carnivorous diet, eating mostly insects, the new population had adapted to eating plants. When they dissected the lizards, scientists found that this new diet required a very differently structured digestive tract, one that now includes structures called cecal valves that allow fermentation of the plant material in the lizard's gut. Such structures had never been observed in *P. sicula* before. Other morphological adaptations included a differently shaped head, that was larger and wider, to allow them to bite off and chew leaves more effectively. This example gives credibility to the theory of punctuated equilibrium.

Exercises

7 Contrast geographical isolation and temporal isolation.

8 In a population of 278 mice, 250 are black and 28 are brown. The alleles are B = black and b = brown. Fill in Table 10.9.

Allele frequencies	Recessive b	q	
	Dominant B	p	
Genotype frequencies	Homozygous recessive bb	q^2	
	Heterozygous Bb	$2pq$	
	Homozygous BB	p^2	

Table 10.9

9 Define polyploidy and give two examples.

Practice questions

1 When do chiasmata form in meiosis?

A During prophase I.

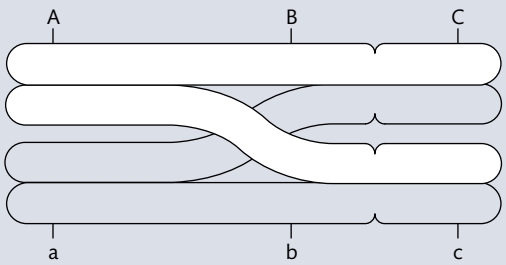
B During metaphase I.

C During anaphase I.

D During prophase II.

(*Total 1 mark*)

2 The diagram below shows a pair of chromosomes during meiosis in a cell in the human testis. The position of the alleles of some genes is indicated.



(a) Deduce, with reasons for your answer, whether the chromosomes are:

(i) autosomes or sex chromosomes (1)

(ii) homologous or non-homologous. (1)

(b) State the stage of meiosis of a cell if it contains pairs of chromosomes as shown in the diagram. (1)

(*Total 3 marks*)

3 In some maize plants the seed is enclosed in a green sheath called a tunica. The allele (T) for this is dominant to the allele (t) for normal, unenclosed seeds. The endosperm of the seed can be starchy (allele E) or sugary (allele e). The genes for these two characteristics are linked. The table below shows the outcome of crosses between a plant heterozygous for both characteristics and one that is homozygous recessive for both characteristics.

Phenotype	Number
Tunica present, starchy	326
Unenclosed seeds, starchy	111
Tunica present, sugary	118
Unenclosed seeds, sugary	295

(a) State the genotype of the heterozygous parent using the correct notation. (1)

(b) Identify which individuals are recombinants in this cross. (1)

(c) Explain what has occurred to cause these results. (2)

(*Total 4 marks*)

4 **(a)** Define the term polygenic inheritance. (1)

(b) Explain, using a named example, how polygenic inheritance gives rise to continuous variation. *(2)*

(*Total 3 marks*)

5 Albinism, a lack of pigmentation in skin and hair, is caused by a recessive allele. Albinism occurs in North America in approximately one in 20 000 persons. Explain how the Hardy–Weinberg equation is applied in this example.

(*Total 5 marks*)

6 Outline ideas about the pace of evolution according to gradualism and punctuated equilibrium.

(*Total 2 marks*)

11 Animal physiology

The male elephant seal is an example of a marine mammal. Marine mammals, at first glance, do not appear to be related to many other easy to recognise mammals like rabbits and deer. The primary reason for this is that marine mammals, like elephant seals, dolphins, and whales are descended from a terrestrial ancestor. They have undergone many evolutionary adaptations to help them survive in a marine environment, but have not lost their integral mammalian characteristics such as hair, mammary glands, and endothermy (being 'warm blooded').

Essential ideas

11.1 Immunity is based on recognition of self and destruction of foreign material.

11.2 The roles of the musculoskeletal system are movement, support, and protection.

11.3 All animals excrete nitrogenous waste products, and some animals also balance water and solute concentrations.

11.4 Sexual reproduction involves the development and fusion of haploid gametes.

Have you ever wondered how similar you are to some of the creatures around you? Does your heart look and work like that of the elephant seal pictured? Do your kidneys function in the same way as the kidneys in a small animal, like a rabbit? How much difference in structure and function is there between animals? The answers are often dependent on evolutionary relationships, but the simplistic answer is frequently 'not much difference'. You and the elephant seal are both mammals. As mammals, you share many similarities in internal organ structure and function. Two animals that are both vertebrates, but not the same class of vertebrates, would be expected to show less similarity in their internal organs. There will be even less similarity when comparing a vertebrate with a non-vertebrate. This chapter will discuss a range of physiological processes of both humans and some of our closer vertebrate relatives.

11.1 Antibody production and vaccination

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Consider ethical implications of research: Jenner tested his vaccine for smallpox on a child.

Understandings:

- Every organism has unique molecules on the surface of its cells.
- Pathogens can be species-specific although others can cross species barriers.
- B lymphocytes are activated by T lymphocytes in mammals.
- Activated B cells multiply to form clones of plasma cells and memory cells.
- Plasma cells secrete antibodies.
- Antibodies aid the destruction of pathogens.
- White cells release histamine in response to antigens.
- Histamines cause allergic symptoms.
- Immunity depends upon the persistence of memory cells.
- Vaccines contain antigens that trigger immunity but do not cause the disease.
- Fusion of a tumour cell with an antibody-producing plasma cell creates a hybridoma cell.
- Monoclonal antibodies are produced by hybridoma cells.

Applications and skills:

- Application: Smallpox was the first infectious disease of humans to have been eradicated by vaccination.
- Application: Monoclonal antibodies to HCG are used in pregnancy test kits.
- Application: Antigens on the surface of red blood cells stimulate antibody production in a person with a different blood group.
- Skills: Analysis of epidemiological data related to vaccination programmes.

Guidance

- *Limit the immune response to mammals.*

Fundamentals of the immune response

A pathogen is any causative agent of disease. The most common pathogens are certain viruses and bacteria. Other organisms, such as certain protists, fungi, and worms, can also be pathogens.

When a pathogen enters the body of a mammal, the immune system responds and attempts to eradicate the potentially disease-causing agent or organism. Unfortunately, the immune system cannot recognize which 'invaders' cause disease (e.g. a virus) and which do not (e.g. a transplanted organ). The recognition is simply one of 'self' or 'not-self'. Each body cell contains the same genetic information, and all body cells have a common set of plasma membrane proteins. Some of the mammalian white blood cells (leucocytes) are capable of recognizing that set of proteins, and consider any cell with those proteins to be 'self'. A virus, bacterium, fungus, or even a transplanted organ, has different (plasma membrane) proteins and thus is recognized as 'not-self'. The term antigen is used for any molecule that is recognized as 'not-self'.

One example of the difference in plasma membrane proteins is the different proteins found on the plasma membranes of human red blood cells (erythrocytes). These plasma proteins determine your blood type. In reality, everyone has two blood types, one that is called the ABO blood type and one that is called the rhesus (Rh) blood type. The ABO blood type is based on the presence or absence of two proteins called the A protein and the B protein. The Rh blood type is based on the presence or absence of a protein called the Rh protein.

The Rh protein was first discovered in rhesus monkeys, hence its name.

The blood type patterns for the ABO blood type are:

- a person who has only the A protein is type A
- a person who has only the B protein is type B
- a person who has both the A and B protein is type AB
- a person who has neither A or B protein is type O.

The blood type patterns for the Rh blood type are:

- a person who has the Rh protein is Rh positive
- a person who does not have the Rh protein is Rh negative.

Later in this chapter you will learn more specifically about what happens during a transfusion if a person receives a blood type that is not compatible with his or her own. In many ways, your immune system treats the incompatible blood just as if it was a virus or bacterium.

False-colour scanning electron micrograph (SEM) showing many macrophages that have identified this parasitic nematode as not-self. The macrophages are attempting phagocytosis of the worm.

The steps of the mammalian immune response

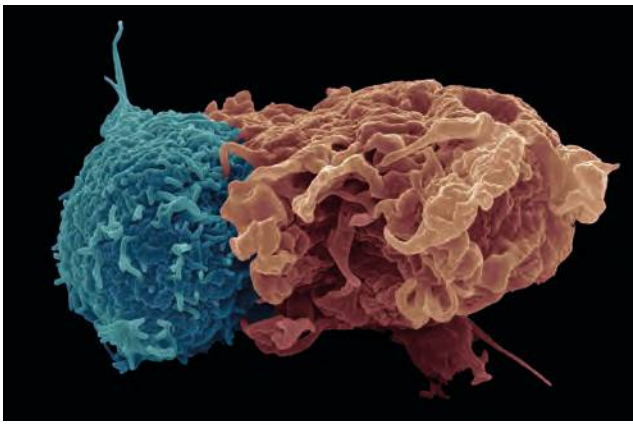
Among the leucocytes in mammalian blood are many different types of B lymphocytes. Each type of B lymphocyte (or plasma cell) is capable of synthesizing and secreting a specific antibody that binds to a specific antigen. The problem is that mammals do not have enough of each type of B cell for the amount of antibody secretion that may be needed. Leucocytes represent roughly 1% of all the cells in the bloodstream, so no one type of B lymphocyte is found in high numbers. Instead, there are cellular communication methods that lead to the cloning (many mitotic cell divisions) of the appropriate B-cell type to combat a specific antigen when needed.

The first type of leucocyte to encounter an antigen is usually the large phagocytic cell known as a macrophage. When a macrophage encounters a not-self antigen found on a pathogen, it engulfs the

pathogen by phagocytosis and partially digests it. Molecular pieces of the invader are displayed on the cell membrane of the macrophage: this is known as antigen presentation. In the bloodstream, leucocytes known as helper T cells can chemically recognize the antigen being presented and become activated.

Helper T cells turn the immune response from non-specific (not-self) to antigen-specific, because the identity of the antigen has now been determined. Helper T cells chemically communicate with (activate) the specific B-cell type that is able to produce the antibody needed. Thus the line of communication is:

Macrophage presents antigen → helper T lymphocyte becomes activated → B lymphocyte becomes activated



SEM showing a macrophage (red) chemically communicating the identity of a pathogen to a helper T cell (blue). This step in the immune response is the beginning of the antigen-specific immune response leading to antibody production.

When a helper T cell activates a specific B cell, the activated B-cell begins a series of cell divisions. This is known as cell cloning because all the daughter cells of these mitotic divisions are capable of producing the same antibody. There are two types of cloned B cells:

- plasma cells, which secrete antibodies immediately and help to fight off the first (or primary) infection
- memory cells, which do not secrete antibodies during the first infection, but are long-lived cells that remain circulating in the bloodstream waiting for a subsequent (or secondary) infection.

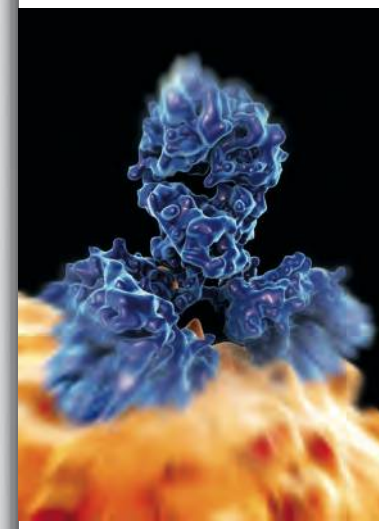
True immunity

Antigen presentation, T- and B-cell activation and B-cell cloning are the events that occur during a primary infection. The mammalian immune system helps eradicate the pathogen, but it cannot protect the body from the pathogen completely because the steps of the primary immune response take time. During that time, the pathogen is producing symptoms associated with the particular disease. How serious those symptoms are largely depends on the speed of replication of the pathogen and the type of tissue the pathogen is damaging.

When a second infection of the same pathogen occurs, the memory cells that were produced during the primary infection are still circulating in the bloodstream. These very long-lived cells, now in relatively large numbers, are capable of responding to the same pathogen very quickly, and with even more antibodies produced, compared with the primary infection.

What are antibodies and how do they help destroy pathogens?

Antibodies are protein molecules produced by plasma cell leucocytes in response to a specific pathogen. Most antibodies are quite similar to each other from a molecular perspective. They are 'Y'-shaped proteins that share many of the same amino acid sequences. At the ends of the forks of the 'Y' are two sequences of amino acids that are unique to each type of antibody. These two areas are called the binding sites of the antibody. The two binding sites of each antibody are identical to each other and are capable of binding to the same type of antigen.



Artwork showing a single antibody molecule. The upper two arms contain identical binding sites for a single antigen.

Humans sometimes make use of antibody production by other animals. Snake antivenom contains antibodies that agglutinate (clump together) the proteins making up the venom of a particular species of snake. The antivenom is made by injecting a horse, goat, or sheep with a small amount of the snake's venom, in order to stimulate antibody production. Later, antibodies are purified from the animal's blood plasma and then used to make the antivenom.

Antibodies help the immune response in several ways. One way is by binding to the pathogen and 'marking' it for destruction by other cells of the immune system. Another way is for antibodies to use their two binding sites on two antigens. This helps by binding the antigens together, often leading to clumps of pathogens because the antibodies are acting to stick them to each other. This agglutination of pathogens helps macrophages and other phagocytic cells find the pathogens for destruction. Another way that antibodies help is by recruiting other cells and proteins to fight the pathogen.

Let's return to the transfusion of blood and blood types. As an example, if a person with a blood type of A Rh^+ (often referred to as A^+ for short) was to donate blood to an individual that has B Rh^- (B^-) blood, the recipient would be receiving erythrocytes that have two types of proteins that they do not already have as part of their genetic makeup. The two proteins would be treated as antigens by the recipient, and antibodies would be created that are specific to the A and Rh proteins on the transfused blood cells. The severity of symptoms as a result of this immune response varies depending on several factors, but is frequently serious as often the patient is receiving blood because of an already severe medical situation.

CHALLENGE YOURSELF

1 During blood transfusions, a person receiving blood cannot receive blood that has one or more of the three erythrocyte plasma proteins that they do not already have on the plasma membrane surface of their own erythrocytes.

State whether each of these transfusions can be safely accomplished, and give the logic behind your answer.

(a) Can a person who has blood type AB Rh^+ receive blood from someone who is O Rh^+?

(b) Can a person who has blood type A Rh^- receive blood from someone who is O Rh^+?

(c) Can a person who is blood type O Rh^+ receive blood from someone who is O Rh^-?

How does a vaccine confer immunity without resulting in symptoms?

One of the fundamental principles of immunity is that an organism cannot be immune to a pathogen before being exposed to it at least once. For some diseases, like a cold, we simply wait for the exposure, experience the symptoms, and then develop the immunity. You will probably not develop symptoms to the same cold virus ever again, but you probably will get another cold as a result of a different cold virus to which you have not yet been exposed.

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In 1796, Edward Jenner administered the very first vaccine. Jenner had noticed that people who milked cows almost never contracted the very serious disease smallpox. He knew that cows developed a similar disease called cowpox. Jenner took a small amount of pus from a cow's cowpox wound and inserted it into a small incision on the arm of an 8-year-old boy. Jenner was taking a chance that something related to the cowpox was conferring immunity to those who worked daily with cows. Jenner later vaccinated more children in the same way, including his own son. His idea worked, because all of the children proved to be immune to smallpox. Despite his success, the lack of ethical perspective and blatant lack of safety measures would not be considered acceptable practice today.

For many diseases, we have developed vaccines that act as the first exposure to the pathogen. A vaccine is typically composed of the chemical components of a pathogen after eliminating the disease-causing abilities of the pathogen. The leucocytes responsible for the primary immune response still recognize the chemical components as antigen(s) and as not-self. Thus the primary immune response takes place. This includes the formation of memory B cells capable of producing antibodies very quickly if there is a later infection with the real pathogen.

The word vaccine comes from the Latin 'vacca', meaning cow.

A vaccination does not prevent an infection but, on subsequent exposure to the real pathogen, the secondary immune response is quicker and results in higher antibody production compared with the primary immune response (see Figure 11.1). After vaccination, most animals respond so quickly to the real pathogen that only very mild symptoms or perhaps no obvious symptoms at all are presented.

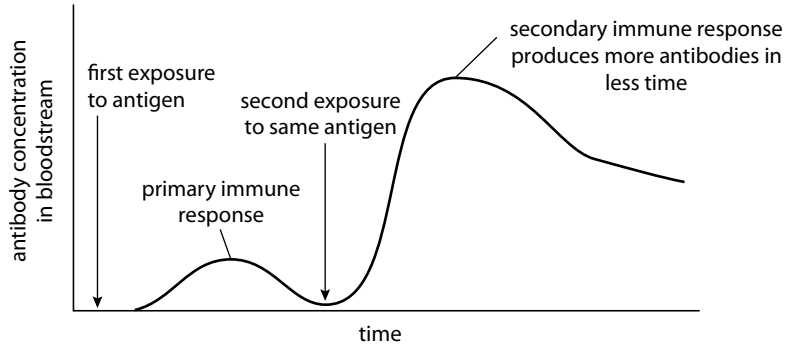


Figure 11.1 Graph showing antibody production in a primary and secondary immune response. The secondary immune response may occur soon after the primary response, or occur months or years after it.

Smallpox was a deadly global disease prior to 1967. In that year, the World Health Organization began a campaign to 'vaccinate the world' against smallpox. That effort was successful after only 10 years, and smallpox became the first disease to be eliminated by humans. The last reported case of smallpox was in Somalia, Africa, in 1977. People are no longer routinely vaccinated against smallpox because there is no one left to transmit the virus.

To learn more about blood transfusions and blood types go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.1.

Worked example

The use of antibodies against antigens is often classified as either passive or active immunity. If the organism benefiting from the antibodies is the same organism that produces the antibodies, then the process is active immunity. Conversely, if one organism produces the antibodies that another organism benefits from, the process is passive immunity.

First identify whether each of the following situations represents passive or active immunity and then 'verbalize' your justification for each decision for each situation.

1. A child is given a vaccine called MMR (measles, mumps, rubella) to confer protection from those three diseases.
2. A newborn child gains protection from antibodies in the first breast milk, called colostrum.
3. A man is injected with an antivenom after being bitten by a poisonous snake.

Solutions

1. Active immunity. The child will use his or her own primary immune response in order to produce both antibodies and memory cells for each of these three pathogens. The pathogens are being injected in a form that will not result in the disease(s) but will trigger the same immune response as the pathogen.
2. Passive immunity. As the newborn is not producing the antibodies, this is passive immunity. Passive immunity confers only short-term effects because memory cells will not be formed for long-term immunity.
3. Passive immunity. The antibodies this man is receiving from the antivenom have been produced by a livestock animal and purified into a form that can be injected into a snakebite victim for immediate benefit. This situation is interesting, however, as the original venom delivered from the snake will result in some degree of long-term immunity because memory cells will result from that active immune response. The active immune response may take too long for a snakebite victim to benefit, thus antivenom provides the immediate protection.

Diseases that cross from one species to another

- HIV/AIDS
- Ebola
- SARS
- H1N1.

The above list of viruses and/or the diseases that they cause all have something in common, other than having serious and potentially deadly symptoms. They have all originated in one species and made the transition to infect another species, specifically infecting human beings. Thankfully, this viral transition from one species to another does not happen very often in nature. The conditions and opportunity must be just right for this to occur. Among the many other conditions that must be met, a protein to protein 'match' must occur for a virus to recognize a cell as a host cell. It is thought that, if two species are in close contact for an extended period of time, long enough for many viral mutations to occur, one of those viral mutations allows the virus to enter a new type of host.

It is more common for diseases resulting from bacteria and fungi to cross species barriers. The following human diseases are problematic in other animals in some form: tuberculosis (caused by a bacterium), salmonella (bacterium) and ring worm (fungus).

There are billions upon billions of viruses, bacteria, and other microbes in our world. Life on Earth is dependent on the fact that only a very small percentage of the total number of microbes is pathogenic to any one species.

Production of monoclonal antibodies

A primary immune response by an organism is called a polyclonal response. This is because the pathogen is typically being recognized as many antigens and not just one. For example, the capsid (protein coat) of a virus is made up of several different kinds of protein. Each of the protein types can cause an immune response, and thus several different kinds of plasma B cells undergo clonal selection, so several different kinds of antibodies are produced. Once a polyclonal immune response has occurred, it is very difficult to separate the different kinds of antibodies that have been produced.

Researchers have developed a clever and unique procedure for forming many antibodies all of the same type. These 'pure' antibodies are called monoclonal antibodies.

The procedure for producing monoclonal antibodies begins with the injection of an antigen into a laboratory animal such as a mouse (see Figure 11.2). The choice of the antigen is very important because the antibodies that will be produced will bind only to this specific antigen. After the injection, the animal is given time to go through a primary immune response. After an appropriate period of time, the spleen of the animal is 'harvested' in order to obtain many blood cells. At least some of the leucocytes cloned for the antigen that was recently injected will be a part of the cellular population within the spleen. There are two problems that need to be addressed at this point in the procedure:

- keeping the B-cell types alive for an extended period of time
- identifying the B-cell type that produces the antibody that recognizes the desired antigen.

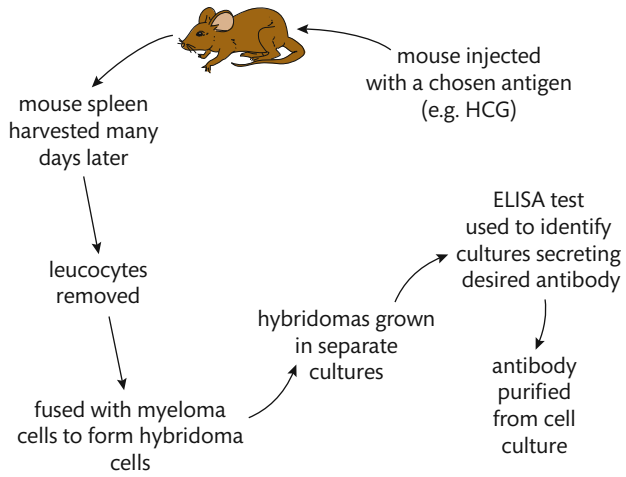


Figure 11.2 A flowchart summarizing the production of monoclonal antibodies.

The B cells are kept alive by fusing them with cancerous (myeloma) cells. When B cells and myeloma cells are grown together in the proper environmental conditions, a few of the cells fuse together and become a hybrid cell called a hybridoma. These hybrid cells have characteristics of both cells: they produce antibodies of a particular type and they are very long-lived (as are all cancer cells). The entire mix of cells is now transferred to an environment in which only the hybridoma cells can survive, and all of the B cells and myeloma cells that did not fuse die.

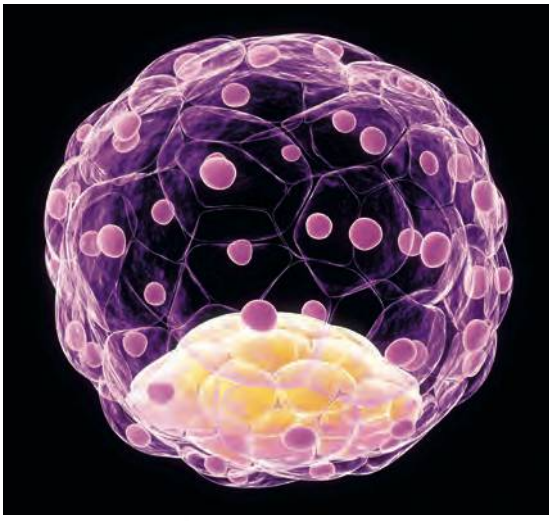
Individual surviving hybridoma cells are now cultured in separate containers. Each container is tested for the presence of a particular antibody. The typical protocol to test for a specific protein (such as an antibody) is called an enzyme-linked immunosorbent assay (ELISA). An ELISA identifies which containers hold a pure colony of B cells that are producing the type of antibody desired. These cells can be cultured for a very long period of time because they have some of the characteristics of a tumour cell. In other words, the hybridoma cells are virtually immortal as long as they are kept in a suitable environment.

Use of monoclonal antibodies to diagnose pregnancy

Monoclonal antibodies can be used for a wide variety of diagnostic purposes. One common use is pregnancy testing. Early in pregnancy, the embryo begins to produce a hormone called human chorionic gonadotropin (HCG). Because HCG is a hormone produced by the embryo, only pregnant women have this hormone; the hormone shows up in small amounts in the bloodstream and urine.

Hybridoma cells can be formed that produce antibodies specific for HCG. These anti-HCG antibodies are chemically bonded to an enzyme that catalyses a colour change when the antibody encounters HCG molecules. This is why pregnancy test results involve a colour indicator.

Computer artwork showing a mammalian embryo at a stage called the blastocyst. In humans, it is at about this stage of development that a pregnancy test detecting HCG could be used successfully. The body of the embryo will develop from the mass of tissue shown in yellow. The remaining cells will help to form the placenta and umbilical cord structures.



Allergies are the result of an immune response releasing histamine

You have seen that the immune system of humans and other animals is designed to protect us from pathogenic 'invaders'. However, there are

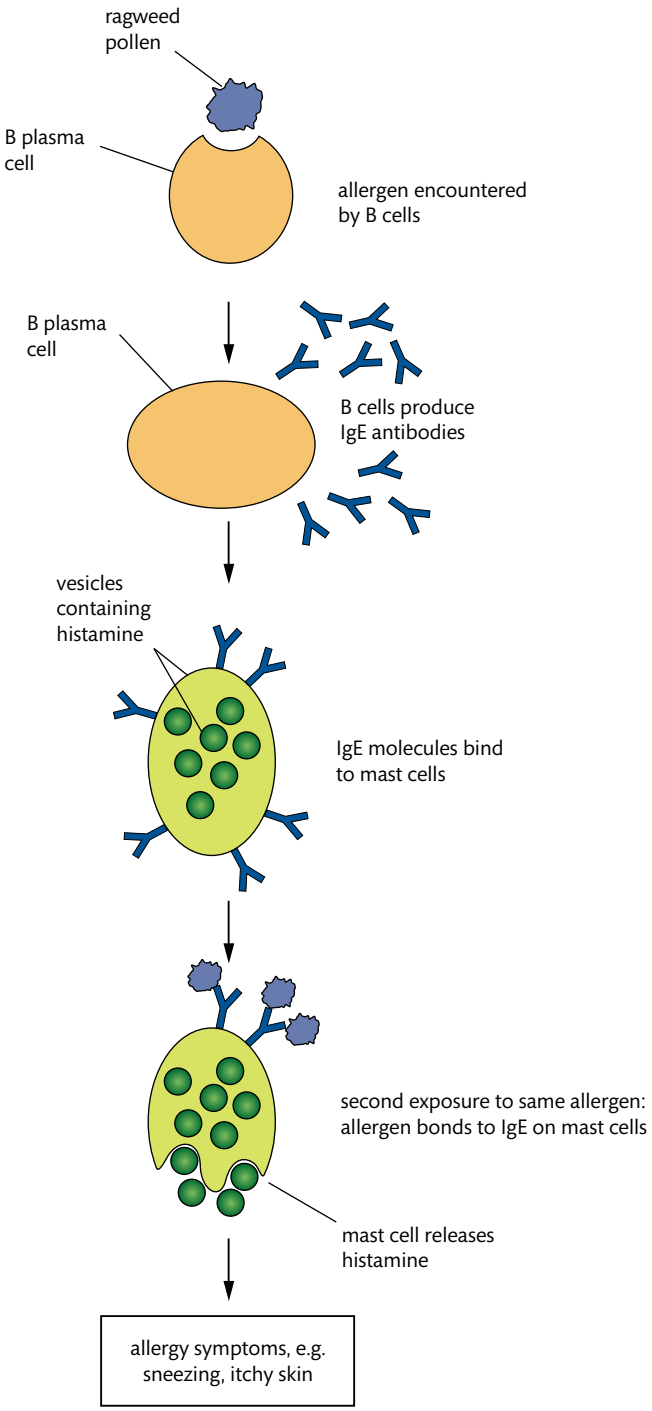


Figure 11.3 An allergic reaction to an allergen.

instances when the immune system itself can create problems. One of those instances is an allergic response. An allergic response occurs when a non-pathogenic substance, called an allergen, is encountered by certain leucocytes. Typical examples of these harmless substances are pollen, peanuts, egg whites, and bee venom, to name just a few. When a person or animal that has a particular allergy is first exposed to that allergen, they produce a particular class of antibody known as IgE. These antibodies then bind to specific white blood cells called mast cells. When the allergen is encountered a second time, these IgE antibodies bind to the allergen and trigger a response that leads to the mast cell releasing large amounts of a chemical called histamine. Histamine causes the symptoms characteristic of an allergy: congestion, sneezing, itchy skin, red skin blotches, and other symptoms, including some that can be quite serious.

Exercises

1. What is the importance of memory cells to an animal's immunity?
2. What are hybridoma cells and what two beneficial characteristics do hybridoma cells possess?
3. How was smallpox completely eradicated by a global vaccination programme?
4. In an emergency situation, a trauma patient can be given blood plasma without first typing his or her blood for a match. How is this possible?

After studying the mechanism by which an allergen leads to the release of histamine molecules causing allergy symptoms, you can better appreciate why allergy sufferers often take antihistamines to alleviate symptoms.

When you study a process such as how allergy symptoms develop, you do not know the process well until you can 'verbalize' the information without written guidance.

To learn more about allergic reactions and histamine, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.1.

11.2 Movement

Understandings:

- Bones and exoskeletons provide anchorage for muscles and act as levers.
- Synovial joints allow certain movements but not others.
- Movement of the body requires muscles to work in antagonistic pairs.
- Skeletal muscle fibres are multinucleate and contain specialized endoplasmic reticulum.
- Muscle fibres contain many myofibrils.
- Each myofibril is made up of contractile sarcomeres.
- The contraction of the skeletal muscle is achieved by the sliding of actin and myosin filaments.
- ATP hydrolysis and cross bridge formation are necessary for the filaments to slide.
- Calcium ions and the proteins tropomyosin and troponin control muscle contractions.

Applications and skills:

- Application: Antagonistic pairs of muscles in an insect leg.
- Skill: Annotation of a diagram of the human elbow.
- Skill: Drawing labelled diagrams of the structure of a sarcomere.
- Skill: Analysis of electron micrographs to find the state of contraction of muscle fibres.

Guidance

- *Elbow diagram should include cartilage, synovial fluid, joint capsule, named bones, and antagonistic muscles.*
- *Drawing labelled diagrams of the structure of a sarcomere should include Z lines, actin filaments, myosin filaments with heads, and the resultant light and dark bands.*
- *Measurement of the length of sarcomeres will require calibration of the eyepiece scale of the microscope.*

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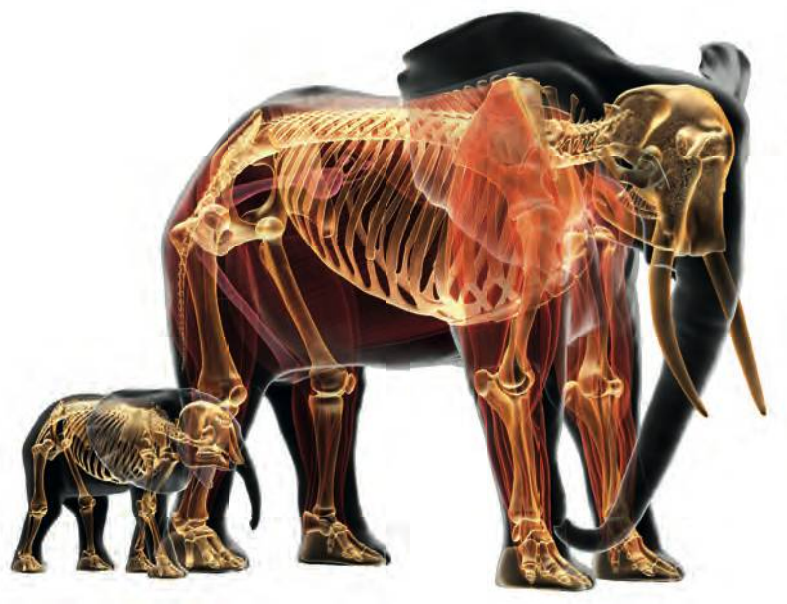
Developments in scientific research follow improvements in apparatus: fluorescent calcium ions have been used to study the cyclic interactions in muscle contraction.

Endoskeletons and exoskeletons



Vertebrates, such as this African elephant, *Loxodonta africana*, and baby, are well known for their internal skeleton, known as an endoskeleton.

The skeleton of an animal provides support. In addition, skeletons provide attachment points for muscles. This is where the term skeletal muscle comes from. When someone hears the term skeleton, they naturally think of the many bones characteristic of most vertebrate animals. These internal bones comprise what is called an endoskeleton. Many animals, like insects, have another kind of skeleton called an exoskeleton. As the name implies, it is a skeleton that is found on the outside of the animal; it is made of a material called chitin rather than bone. Like an endoskeleton, an exoskeleton also provides support and attachment points for muscles. The attachment points for muscles are found on the outside of the bones of an endoskeleton, and on the inside of an exoskeleton. Many individual bones and segments of exoskeletons also act as levers, to maximize efficiency for a variety of movements. The evolution of animals with exoskeletons includes species with leverage potential that can give rise to incredible feats of strength and jumping ability. An excellent example is the

Asian weaver ant, *Oecophylla smaragdina*; these ants have been shown to be able to lift over 100 times their own body mass. Another example is the flea, *Ctenocephalides felis*, that infects household cats; these fleas are known to jump over 150 times their own body length.

A grasshopper uses exoskeleton joint leverage coupled with a catapult mechanism to jump about 1 m. If you scale the size of a grasshopper up to an organism the size of a human, that jump would be about 40 m.

To learn more about grasshoppers' legs, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.2.

The immovable attachment point of a muscle is called its origin; the movable attachment point is called the insertion.

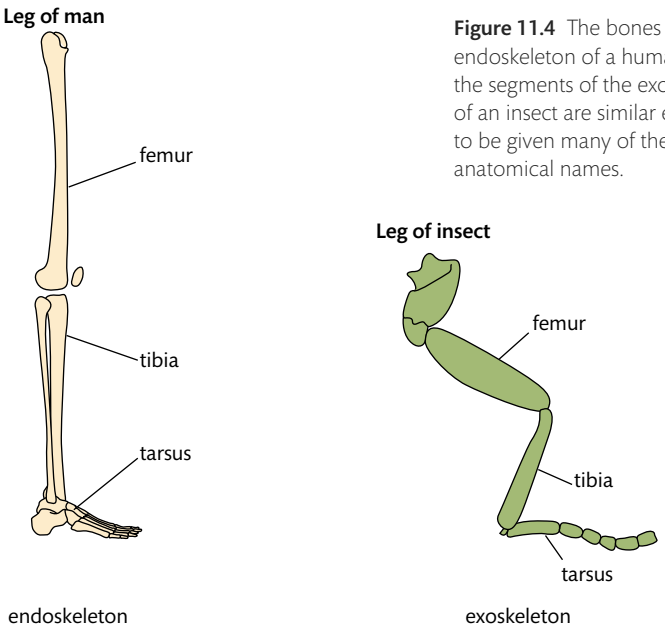


Figure 11.4 The bones of the endoskeleton of a human and the segments of the exoskeleton of an insect are similar enough to be given many of the same anatomical names.

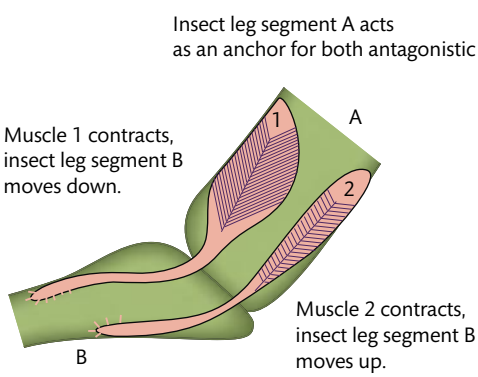


Figure 11.5 The segments of an insect's leg are moved up and down by the action of antagonistic muscle pairs.

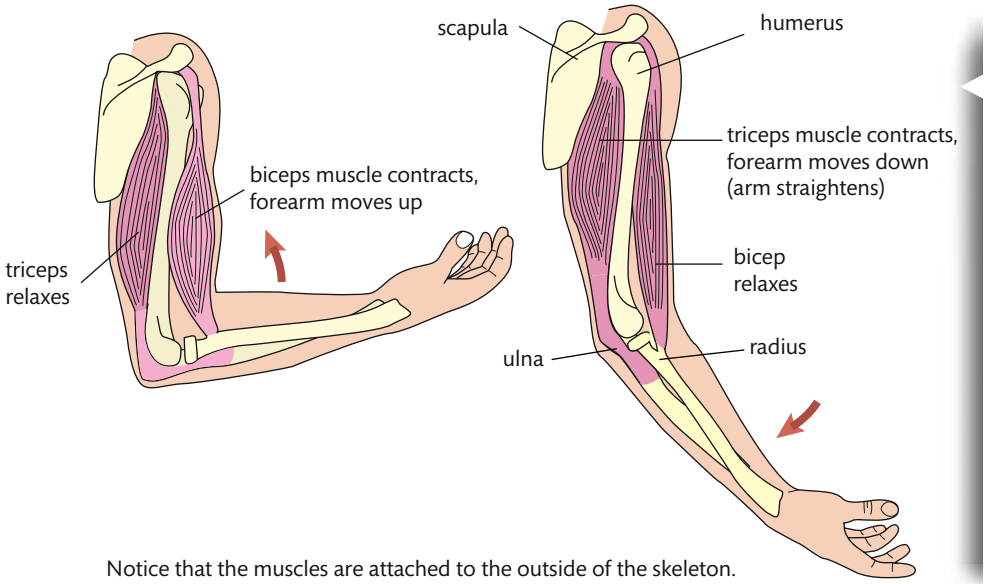
Muscles work in antagonistic pairs

When a muscle contracts, one end of that muscle is connected to a bone (or exoskeleton) that is not designed to move. The other end of the same muscle is connected to a bone that is designed to move. Think of the immovable bone as an anchor for the desired movement. Because each muscle can only shorten in order to cause a single movement, muscles must work in pairs so that the opposite movement of the bone can also occur. These pairs of muscles that accomplish opposite movements are called antagonistic pairs. The pair of diagrams in Figures 11.5 and 11.6 show how a joint in an insect and a human move by antagonistic muscle pairs.

Synovial joints provide limited movements

Some joints in the body of mammals are synovial joints. These are bone-to-bone joints where there is a self-contained capsule area that contains a lubricant called synovial fluid. The ends of the bones within the capsule are also coated with cartilage in order to cushion any bone-to-bone contact.

An example of a synovial joint is the human elbow. The elbow is also an example of a hinge joint, which provides an opening-and-closing movement similar to the action of a door. When you hold your arm out from your body and turn your hand so that the palm is sometimes up and sometimes down, you are not using the hinge joint



Notice that the muscles are attached to the outside of the skeleton.

Figure 11.6 The forearm of a human is moved up and down by the action of the triceps and biceps.

of the elbow to do that. Instead, you are moving the two bones of your forearm (radius and ulna) to accomplish the rotation. We have evolved that method of motion because the elbow joint is not capable of such a rotation. The joint where your humerus (upper arm bone) joins to the body at the shoulder is an example of a ball-and-socket joint, which is much freer and can move in various directions compared with the elbow joint. The same pattern exists in your legs, where the hip joint is a freely movable ball-and-socket joint and the knee is a hinge joint.

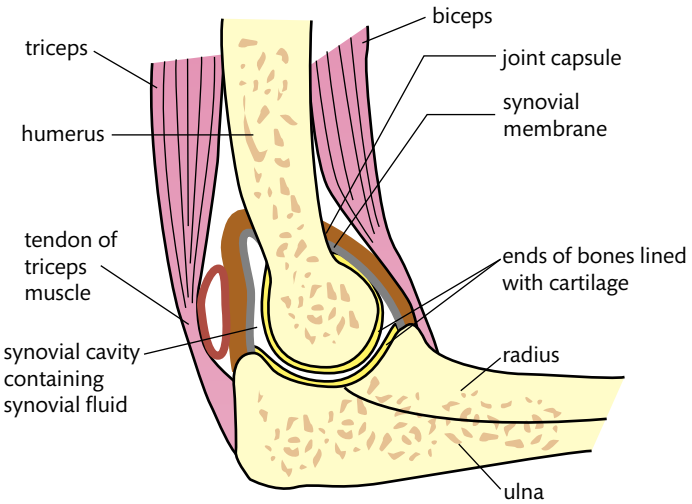


Figure 11.7 The human elbow in section.

Table 11.1 The parts and function of the human elbow joint

Joint part	Function
Cartilage	Reduces friction and absorbs compression
Synovial fluid	Lubricates to reduce friction and provides nutrients to the cells of the cartilage
Joint capsule	Surrounds the joint, encloses the synovial cavity, and unites the connecting bones
Tendons	Attach muscle to bone
Ligaments	Connect bone to bone
Biceps muscle	Contracts to bring about flexion (bending) of the arm
Triceps muscle	Contracts to cause extension (straightening) of the arm
Humerus	Acts as a lever that allows anchorage of the muscles of the elbow
Radius	Acts as a lever for the biceps muscle
Ulna	Acts as a lever for the triceps muscle

A muscle fibre is a muscle cell

Like any other tissue in the body, muscle is made up of cells. There are three kinds of muscle tissue: smooth muscle, cardiac muscle, and skeletal (striated) muscle. We will be considering the structure and action of skeletal muscle. These cells are highly modified for contraction, and thus their cellular structure is not as apparent as in many cells. Each muscle is composed of thousands of cells, called muscle fibres because of their elongated shape. Muscle tissue also includes surrounding connective tissues, blood vessels, and nerves.

Muscle fibres (cells) contain multiple nuclei that lie just inside the plasma membrane, which is called the sarcolemma. The sarcolemma has multiple tunnel-like extensions that penetrate the interior of the cell. These penetrating invaginations (infoldings) are called transverse tubules or T tubules.

The cytoplasm of muscle fibres is called the sarcoplasm. The sarcoplasm contains large numbers of organelles that store glycogen as an energy reserve. The sarcoplasm also contains a molecule related to haemoglobin, called myoglobin. Myoglobin stores oxygen and only releases that oxygen when muscle tissue is very heavily used and the normal supply of oxygen from haemoglobin becomes limited.

TOK

Some of the earliest detailed studies of human muscles were made by Renaissance artists such as Leonardo da Vinci. da Vinci kept detailed anatomical notebooks about cadavers.

Myofibrils run the length of the cell. There are many myofibrils and they are parallel to one another. Numerous mitochondria are found packed between the myofibrils in order to supply the adenosine triphosphate (ATP) necessary for muscle contraction. The myofibrils are where the contractile units called sarcomeres are found, dominated by the proteins actin and myosin. The repeating sarcomeres are the reason why striated muscle has a banded pattern.

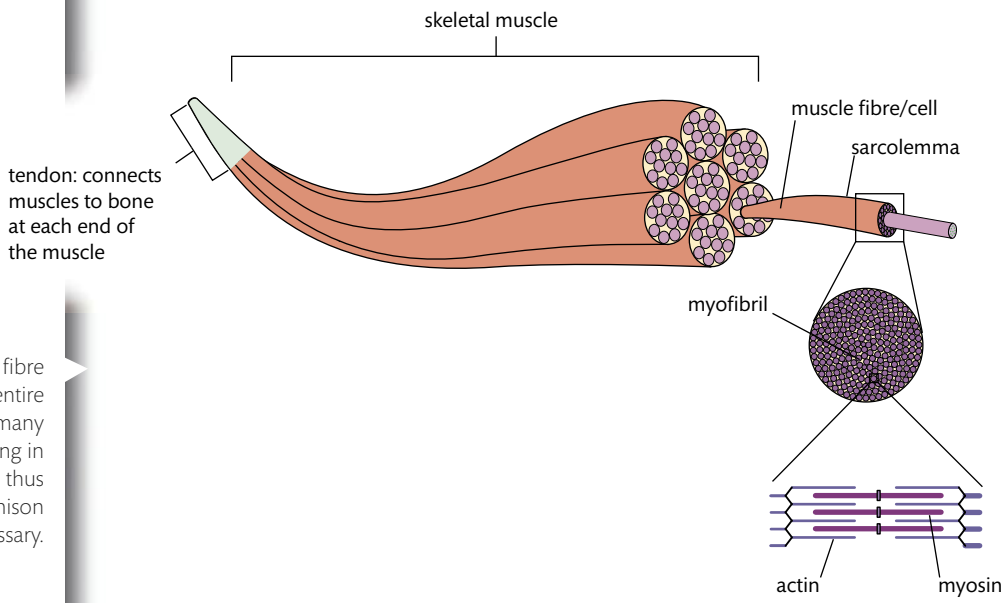
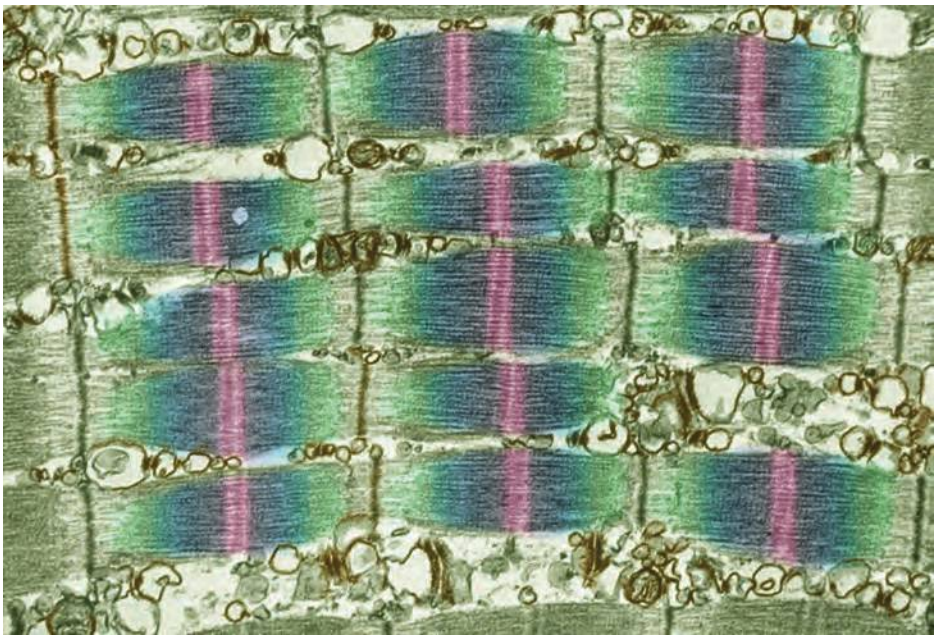


Figure 11.8 Each muscle fibre is a muscle cell. The entire muscle is composed of many muscle fibres all running in the same direction and thus all able to contract in unison when necessary.



False-colour electron micrograph of myofibrils of skeletal (striated) muscle. Striated muscle is the muscle tissue that moves bones. There are a total of five myofibrils visible in this photo, arranged from top to bottom.

The contracting units of myofibrils are sarcomeres

A myofibril is composed of many side-by-side contracting units called sarcomeres. One sarcomere extends from one Z line to the next Z line (see Figure 11.9) It is these repeating sarcomeres that give skeletal muscle its other name, striated muscle. Put very simply, muscle tissue is able to shorten (contract) because each sarcomere gets shorter. Because the sarcomeres are connected to each other, the entire muscle gets shorter. Thus a muscle pulls on a tendon that is connected to a bone (or exoskeleton) and a movement of the skeleton (or exoskeleton) occurs.

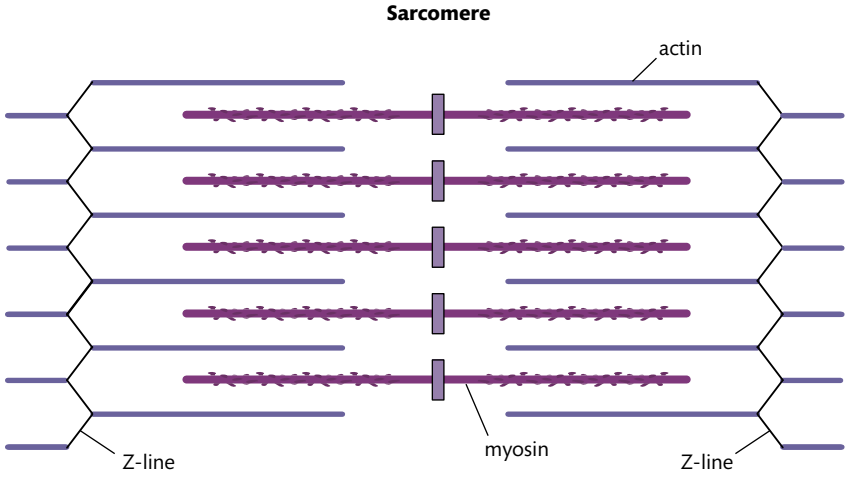


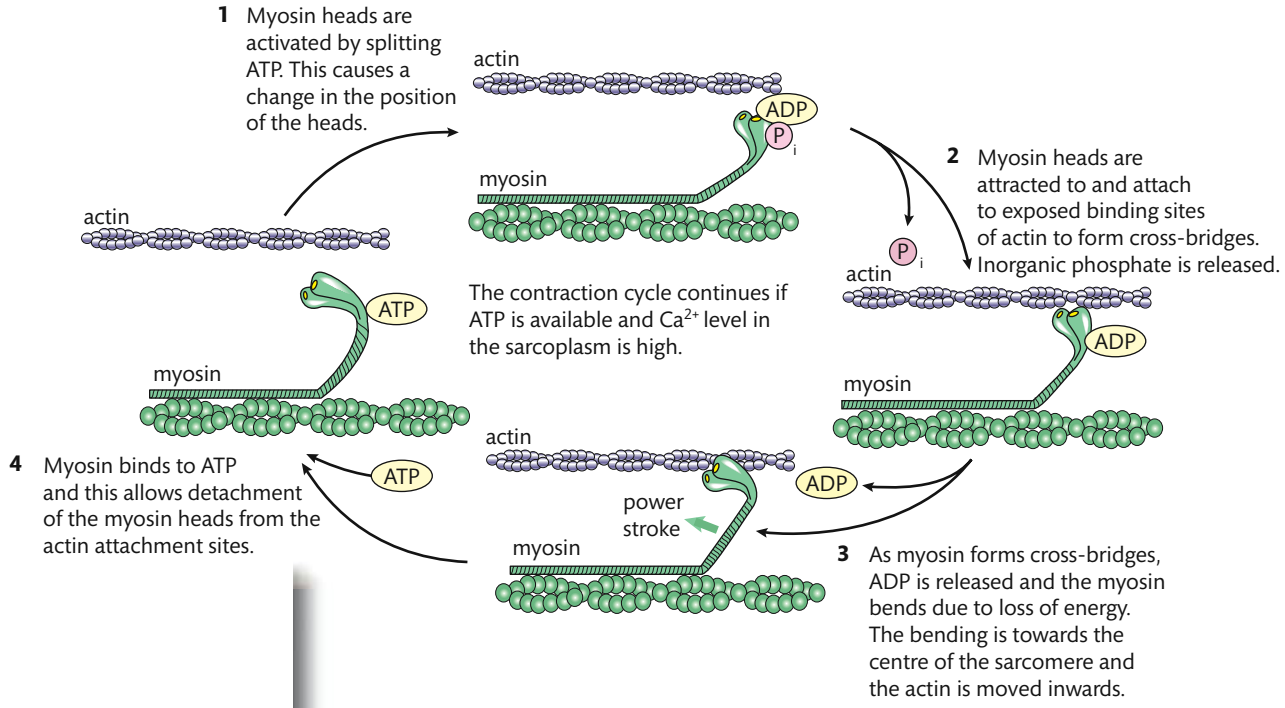
Figure 11.9 The structure of a single relaxed sarcomere. Notice that the Z lines form the ends of this and all sarcomeres. In electron micrographs of myofibrils, the darkest areas are where both actin and myosin are found together. The lightest areas are where only actin is found. Karp 2009.

Notice in the diagram of the sarcomere (Figure 11.9) that myosin fibres are relatively thick with head-like structures, and actin is relatively thin. Both of these fibres are proteins, which explains why, as a source of food, muscle tissue is so rich in protein. Notice also that there is no way that myosin can get any shorter because it is one continuous protein within the sarcomere. Actin, on the other hand, is able to move, each side sliding towards the centre of the sarcomere and thus shortening the entire

Figure 11.10 The activity of one myosin head and one actin fibre illustrate the interaction of all the myosin heads and actin fibres within a single sarcomere.

sarcomere (think of it as the Z lines coming closer to each other). Thus the question becomes: how do the actin filaments of a sarcomere all move toward the centre and collectively make the muscle contract?

The sliding filament theory of muscle contraction



ATP keeps each myosin head ready for action, waiting for an action potential from a motor neurone. Using muscles for exercise uses more ATP, because each use requires a new ATP to prepare the myosin heads for a new cycle of activity to begin.

Many animal's bodies, including humans, become very rigid a few hours after death, a condition called rigor mortis. This condition is the result of no new ATP being generated after death, and thus the myosin heads cannot detach from actin-binding sites, resulting in a rigid muscle condition. Rigor mortis decreases after about 36 hours.

Refer frequently to Figures 11.8, 11.9 and 11.10 when following these steps of a muscle contraction.

- A motor neurone carries an action potential until it reaches the final synapse, called a neuromuscular junction.
- A neurotransmitter called acetylcholine is released into the synaptic gap between the neurone end buttons and the sarcolemma of the muscle fibre.
- The acetylcholine binds to receptors on the sarcolemma.
- Sarcolemma ion channels open and sodium ions (N^+) move through the membrane.
- The resulting action potential moves through the T tubules, causing the release of calcium ions (Ca^{2+}) from the sarcoplasmic reticulum.
- The released calcium ions flood into the sarcoplasm.
- The myosin heads then attach to binding sites on the actin (step 2 of Figure 11.10).
- The myosin heads all flex towards the centre of the sarcomere.
- The entire sarcomere shortens as the Z lines move towards each other (step 3 of Figure 11.10).
- ATP binds to the myosin head, resulting in the detachment of myosin from the actin, and awaits another action potential from a motor neurone (step 4 of Figure 11.10).

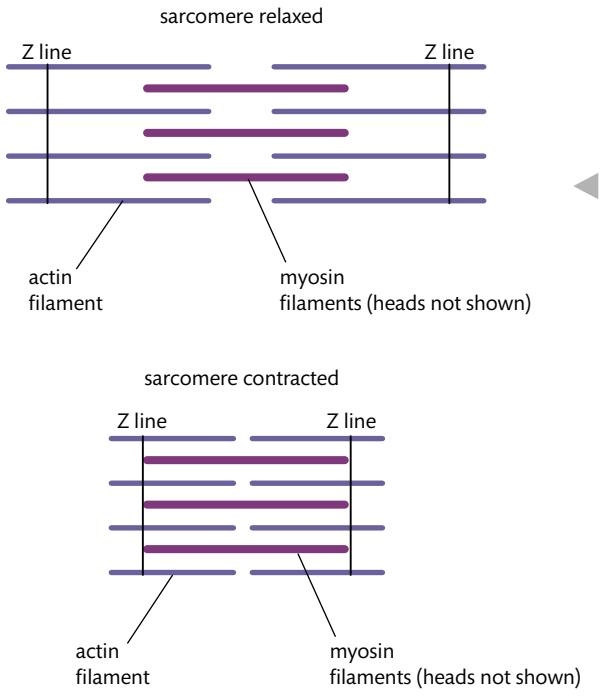


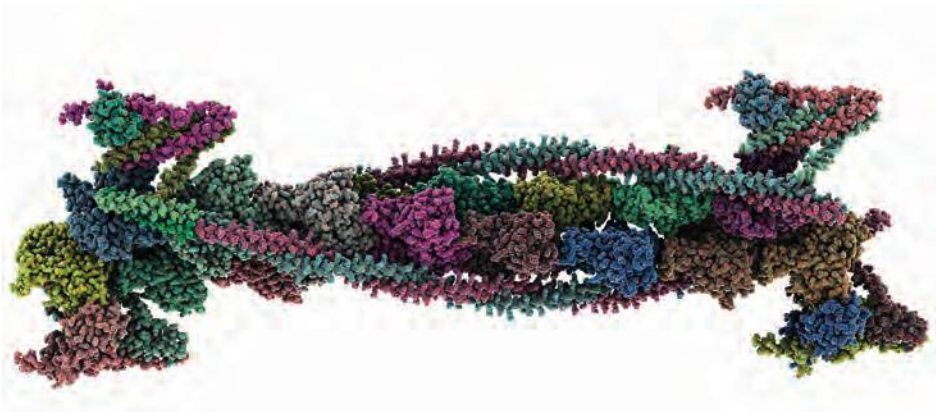
Figure 11.11 A sarcomere shown at rest and during a contraction. Notice that the Z lines in the contracted sarcomere are closer together. There are many sarcomeres in a series, and when all of them become shorter, the entire muscle fibre becomes shorter. This will result in the movement of a bone.

Preparing to be able to draw diagrams from memory in an exam has to involve actually practising drawing them. Simply looking at a diagram will not enable you to draw that diagram.

CHALLENGE YOURSELF

2 Figure 11.11 shows you a simplified diagram of a sarcomere in a relaxed position and when contracted. First study the top diagram of the relaxed sarcomere. With a pencil and blank piece of paper, attempt to redraw and label this diagram. When you have mastered this diagram, study the bottom diagram of the contracted sarcomere and draw it so that the myosin aligns directly with the myosin of the first diagram. The actin fibres will change position so that the Z lines become closer together. Make sure to label the following on each diagram: Z lines, actin filaments, myosin filaments, heads of myosin filaments, area of light bands, and area of dark bands.

To learn more about muscles, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.2.



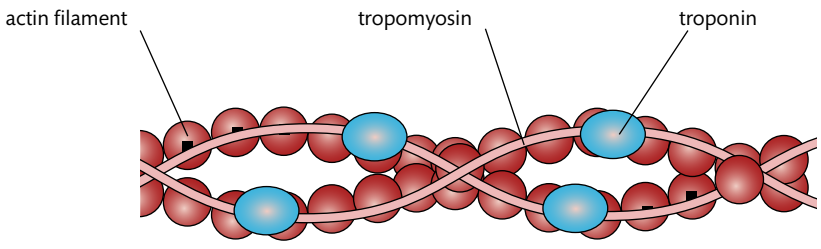
Molecular model showing the complexity of the protein components of muscle tissue. This image shows myosin with 'heads' on both sides. Troponin and tropomyosin, the proteins that help control when a sarcomere contracts are also shown. You will read about those two proteins in the next section.

The role of troponin and tropomyosin during a muscle contraction

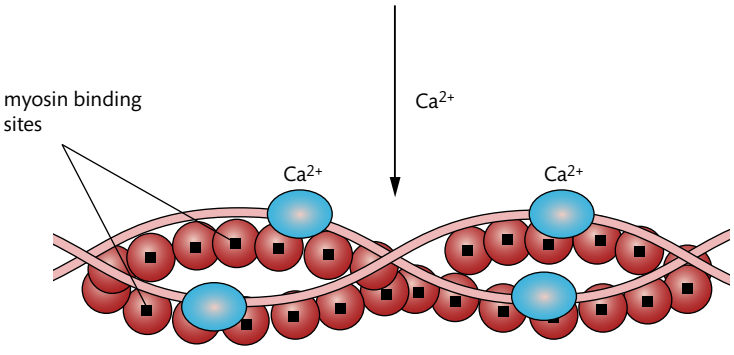
One step of the muscle contraction sequence given above reads: 'The myosin heads then attach to binding sites on the actin.' This important step determines when a muscle contraction occurs, as the binding sites on the actin are not always available. When a muscle is not contracting the binding sites on actin are covered by a thin protein filament called tropomyosin. In addition, another protein called troponin binds to tropomyosin at regular intervals along the length of tropomyosin. Troponin has binding sites for calcium ions (Ca^{2+}). Look again at the step: 'The released calcium ions flood into the sarcoplasm.' Remember that the sarcoplasm is simply the cytoplasm inside the muscle fibre (the cell) and thus the calcium ions are temporarily at a high concentration inside the cell, where many actin filaments are located within the sarcomeres. These calcium ions bind to troponin, which stimulates the tropomyosin filament to slide, uncovering the actin binding sites. Instantly the 'ready to go' myosin heads find an actin binding site and undergo the flex movement of the myosin heads, leading to the shortening of the sarcomere and thus the entire muscle.

Recall the events that led to the influx of calcium ions into the sarcoplasm. Those events were initiated by an action potential from a motor neurone. A motor neurone does not send action potentials randomly; action potentials are only sent when there has been a voluntary 'decision' by the cerebrum to make a movement. Thus the release of calcium ions and the interaction with troponin and tropomyosin represent the link between the nervous system and the muscular system, as well as the skeletal system that is being moved.

A coloured SEM showing a neuromuscular junction. This is a synapse that occurs between a motor neurone and muscle tissue. The motor neurone is the thin filament coming in from above.



tropomyosin fibres block myosin binding sites on actin filaments



Ca^{2+} binding to troponin moves tropomyosin, exposing myosin binding sites

Figure 11.12 A motor neurone has sent action potentials to a muscle, leading to an influx of calcium ions into the sarcoplasm. Myosin heads are energized and ready to connect to actin-binding sites if/when the binding sites become uncovered. http://classes.midlandstech.edu/carterp/Courses/bio110/chap07/chap07.html

Exercises

5 List three common functions that bones of some animals and the exoskeleton of other animals have.

6 Explain why muscles occur in antagonistic pairs.

7 Ca^{2+} in muscle contraction is referred to as a secondary messenger. Why does this make sense?

8 In the human elbow, state what provides the following functions.

(a) Movement of the radius and ulna up towards the face.
(b) Attachment of the bicep to the radius and scapula.
(c) Nearly frictionless movement at the elbow joint.
(d) Movement of the radius and ulna away from the face.

Researchers can follow the role of calcium ions in muscle contraction by using fluorescent calcium ions and specialized imaging technology that allows the fluorescent calcium to be seen and quantified during a contraction sequence.

11.3 The kidney and osmoregulation

Understandings:

- Animals are either osmoregulators or osmoconformers.
- The Malpighian tubule system in insects and the kidney carry out osmoregulation and removal of nitrogenous waste.
- The composition of blood in the renal artery is different from that in the renal vein.
- The ultrastructure of the glomerulus and Bowman's capsule facilitate ultrafiltration.
- The proximal convoluted tubule selectively reabsorbs useful substances by active transport.
- The loop of Henle maintains hypertonic conditions in the medulla.
- ADH controls reabsorption of water in the collecting duct.
- The length of the loop of Henle is positively correlated with the need for water conservation in animals.
- The type of nitrogenous waste in animals is correlated with evolutionary history and habitat.

Applications and skills:

- Application: Consequences of dehydration and overhydration.
- Application: Treatment of kidney failure by haemodialysis or kidney transplant.
- Application: Blood cells, glucose, proteins, and drugs are detected in urinary tests.
- Skill: Drawing and labelling a diagram of the human kidney.
- Skill: Annotation of diagrams of the nephron.

Guidance

- *ADH will be used in preference to vasopressin.*
- *The diagram of the nephron should include glomerulus, Bowman's capsule, proximal convoluted tubule, loop of Henle, distal convoluted tubule; the relationship between the nephron and the collecting duct should be included.*

NATURE OF SCIENCE

Curiosity about particular phenomena: investigations were carried out to determine how desert animals prevent water loss in their wastes.

Nitrogenous waste products and excretion

The blood plasma of an animal is a constantly changing solution. Reactions within body cells are collectively referred to as metabolism. The bloodstream supplies the substances needed for an animal's metabolism, and also removes molecular waste products from the tissues. Given this constant addition of wastes, such as urea, the bloodstream needs to be continuously filtered and cleansed.

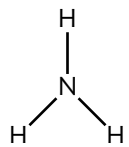
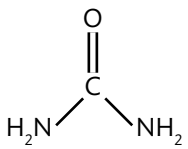
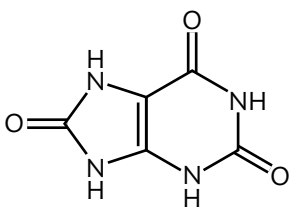
Urea is a waste product from the metabolism of amino acids. The body has no way to store excess amino acids (those not needed immediately for protein synthesis). In many animals, excess amino acids undergo a chemical reaction called deamination. As the name suggests, the amine group of each amino acid is removed. This amine group (NH_2) is incorporated into one of three types of waste molecules: ammonia, urea, or

uric acid. In some animals, including all mammals, it is the job of the kidneys to filter and cleanse the bloodstream of molecules like urea and other molecular wastes. Other animals have different structures that accomplish this same function, such as the Malpighian tubules of insects. However, no matter which organ is involved, excretion is the removal of the waste products of metabolic pathways from the body.

Evolutionary history, habitat, and the type of nitrogenous waste

The waste products that result from the deamination of amino acids are called nitrogenous wastes because each type of waste product contains one or more atoms of nitrogen. There are advantages and disadvantages to each of the three types of nitrogenous wastes. No animal is independent of its evolutionary history: if a species has an ancestral origin that primarily used one of the three nitrogenous waste types, then the emerging species is most likely to use the same nitrogenous waste type. In other words, animals cannot evolve an entirely new physiology even when they undergo enough change to qualify as a new species. Let's look at the advantages and disadvantages of each type of nitrogenous waste.

Table 11.2 The three types of nitrogenous waste

Structure of nitrogenous waste	Example organism	Advantages	Disadvantages
Ammonia 	Fish	Requires very little energy to produce	Very toxic in blood and tissues; must be diluted and removed from the body quickly by using a great deal of water
Urea 	Mammals	Requires less energy to produce compared with uric acid; toxic in blood and tissues but only at physiologically abnormal levels	Requires more energy to produce compared with ammonia; requires some water for dilution and removal from the body
Uric acid 	Birds	Relatively insoluble in aqueous solutions such as blood and cytoplasm; can be stored within specialized structures within some animal's eggs; requires little to no water for dilution and removal from the body	Its complex structure requires a great deal of energy to produce

Let's look at each type of animal mentioned in Table 11.2 and see why each type of nitrogenous waste makes sense for that animal based on its probable habitat and evolutionary history.

Fish use ammonia as their primary nitrogenous waste. Because of their habitat, fish have an unlimited water supply that they can use to dilute and flush out the highly toxic ammonia. They benefit from the fact that this nitrogenous waste is very energy 'inexpensive'.

Mammals produce and excrete urea. As urea is only toxic at relatively high concentrations, mammals can cope with a certain level of urea in the tissues and blood. The system works well as long as the level of urea is kept under control by the constant filtering of the kidneys. Urea, as a component of urine, can be stored temporarily in the urinary bladder. Water is not as accessible to mammals as it is to fish, so mammals have a system that needs less water for dilution and elimination compared with ammonia.

Birds and reptiles both use an egg that is self-contained for nutrients and water for development until hatching. From an evolutionary perspective, this was the big step that separated reptiles and birds from the aquatic environment of their immediate ancestors, the amphibians. A huge problem that had to be solved with this step is that ammonia cannot be stored within the self-contained egg. The evolutionary solution was uric acid. Even though uric acid is quite energy expensive to produce as a nitrogenous waste, it is not water soluble and so can be stored within a specialized structure within the egg as the embryo develops. Adult birds continue to produce uric acid, and this gives them some independence from having to find water frequently; animals that use ammonia or urea as their waste product have to drink relatively frequently compared with birds.

CHALLENGE YOURSELF

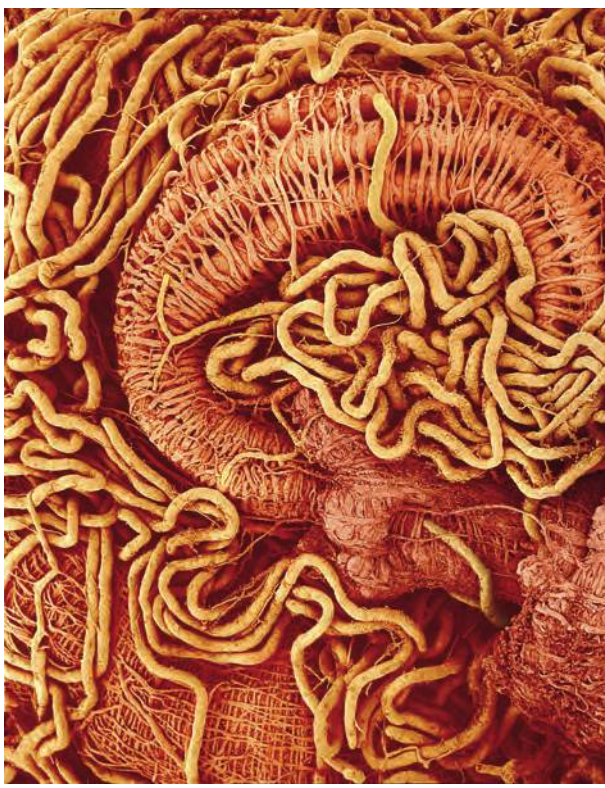
3 What type of nitrogenous waste is produced and excreted by each of the following animals. Provide your reasoning for each answer.
- **(a)** Great white shark
- **(b)** Walrus
- **(c)** Penguins
- **(d)** Marine sea turtles.

Unlike insects, humans and other vertebrate animals use a closed circulatory system. Blood is always contained in blood vessels, with chemical exchanges occurring between capillaries and any fluid surrounding those capillaries.

Nitrogenous waste excretion in insects

Insects have an open circulatory system. This means that their blood is sometimes outside the blood vessels in one or more body cavity. In effect, many of their internal organs are bathed in this blood.

The body cavities of insects have small tubes called Malpighian tubules that lie within the pools of blood within the cavities. These tubes are closed at one end (the distal end) and open into the insect's gut at the other (proximal) end. Various molecular components of the insect's blood enter the Malpighian tubules close to the distal end and then undergo a selective reabsorption process. In effect, nitrogenous wastes, excess water, and many salt ions (Na^+, K^+, Cl^-) remain in the tubules. Useful substances, non-excess water, and unused nutrients from the blood are transported back into the pool of blood of the body cavity. The nitrogenous waste (mainly uric acid) and excess water (if any) move within the Malpighian tubules to the proximal end that empties into the gut. The waste is then eliminated along with the faeces.



A coloured SEM of the body cavity of a honeybee, *Apis mellifera*. The numerous 'worm-like' tubes are Malpighian tubules, and the larger central area is the part of the intestine that the tubules empty nitrogenous wastes and excess water into.

The composition of blood plasma in the renal artery compared with the renal vein is different, and that difference is because of the filtering action of the kidney. The primary difference between the blood in these two vessels is the levels of water, salt ions, and urea.

Anatomy of the kidney

The function of kidneys is to filter waste products from the blood. There is a major blood vessel called the renal artery that takes blood into each of the kidneys. The filtered blood drains away from the kidney by a blood vessel known as the renal vein.

Urine is the fluid produced by the kidneys; it consists of water and dissolved waste products that have been removed from the bloodstream. Urine collects within each kidney in an area called the renal pelvis. The renal pelvis drains this urine into a tube called the ureter, which then takes the urine to the urinary bladder. When the kidney is cut in section, as shown in Figure 11.13, you can see the layer of tissue surrounding the renal pelvis, which is called the renal medulla; the layer to the outside of that is the renal cortex.

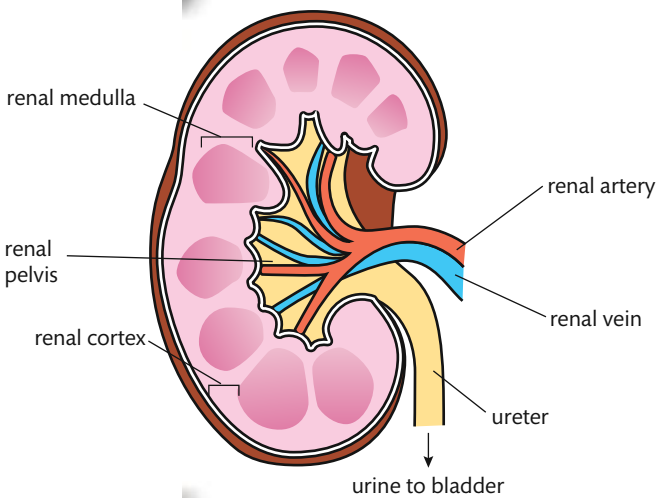
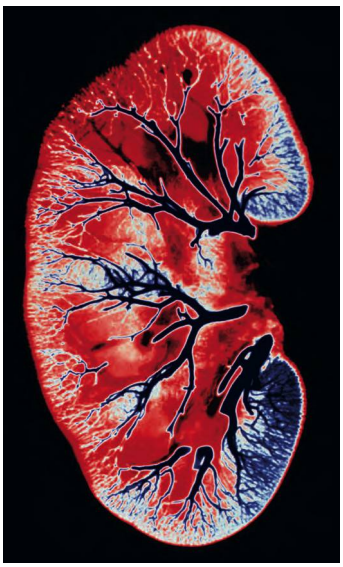


Figure 11.13 Sectioned view of the human kidney.



An angiogram is a form of X-ray where one or more dyes have been added to blood vessels to demonstrate blood flow clearly. This angiogram shows the network of blood vessels within a kidney. The purpose of a kidney is to filter blood, so kidney tissue is highly vascular.

You need to be able to draw and label a diagram of the human kidney. Use Figure 11.13 as a guide, and practise doing just that!

Nephrons are the filtering units of kidneys

Each kidney is made up of about 1.25 million filtering units known as nephrons. Each nephron consists of:

- a capillary bed, called a glomerulus, which filters various substances from the blood
- a capsule surrounding the glomerulus, called the Bowman's capsule
- a small tube (tubule) that extends from Bowman's capsule, consisting of the proximal convoluted tubule, loop of Henle, and distal convoluted tubule
- a second capillary bed, called the peritubular capillary bed, that surrounds the three-part tubule mentioned above.

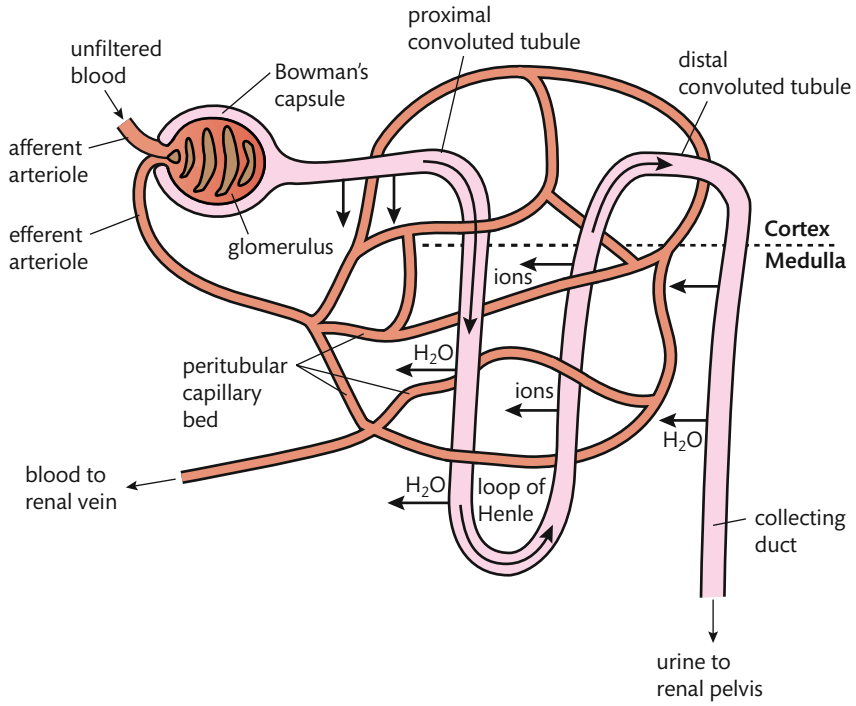


Figure 11.14 A single nephron of the mammalian kidney. Notice the dotted line showing the portion of each nephron in the renal cortex of the kidney and the portion of each that extends down into the renal medulla. Use this diagram to follow the process of urine formation in the next few sections of the text.

You also need to be able to annotate one or more diagrams of a nephron. Study the material concerning nephron function and especially Figure 11.14, and then practise adding function labels to the various parts of the nephron.

Blood is ultrafiltered within Bowman's capsule

Each nephron contains a very small branch of the renal artery known as an afferent arteriole. This brings unfiltered blood to the nephron. Inside the Bowman's capsule, the afferent arteriole branches into a capillary bed called the glomerulus. The glomerulus is similar to most other capillary beds except that the walls of the capillaries have fenestrations (very small slits) that open when blood pressure is increased. The increase in blood pressure is provided by the efferent arteriole, which drains blood from the glomerulus and has a smaller diameter than the afferent arteriole. Connecting a larger diameter blood vessel to a smaller diameter blood vessel creates a higher pressure where they join, at the glomerulus.

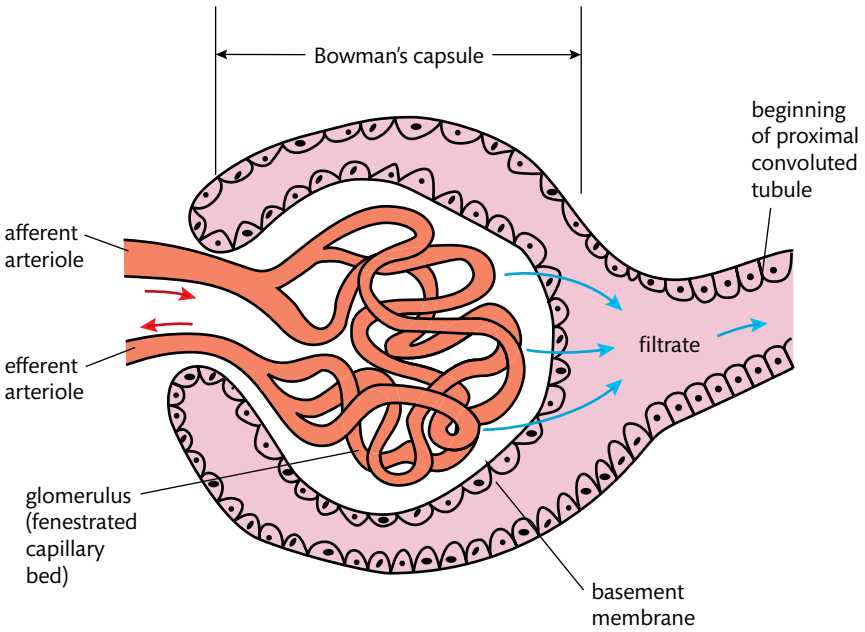


Figure 11.15 Bowman's capsule is the site of the process called ultrafiltration.

Ultrafiltration is the term used to describe the process by which various substances are filtered through the glomerulus (and its fenestrations) under the unusually high blood pressure in this capillary bed. The fluid that is ultrafiltered from the glomerulus passes through the basement membrane, which helps prevent large molecules like proteins from becoming a part of the filtrate. The filtrate then enters the proximal convoluted tubule. The blood that did not get filtered, including all of the cells and proteins as well as many other molecules, exits the Bowman's capsule in the efferent arteriole.

Reabsorption recovers substances that are needed

The filtrate that leaves the Bowman's capsule contains many substances that the body cannot afford to lose as part of the urine. The body needs to keep a great deal of the water, many of the salt ions, and all of the glucose that is in the filtrate. These substances need to be 'rescued' by reabsorption back into the bloodstream. Much of the reabsorption process occurs from the proximal convoluted tubule. Substances leave the tubule filtrate and are taken back into the bloodstream via the peritubular capillary bed. This capillary bed is so named because it surrounds (peri-) the tubule (see Figure 11.14).

The wall of the proximal convoluted tubule is a single cell thick. As you can see in Figure 11.16, the tubule is composed of a single ring of cells. The interior of the resulting tube is called the lumen and the filtrate flows within this lumen. The inner portion of each of the tubule cells has microvilli in order to increase the surface area for reabsorption.

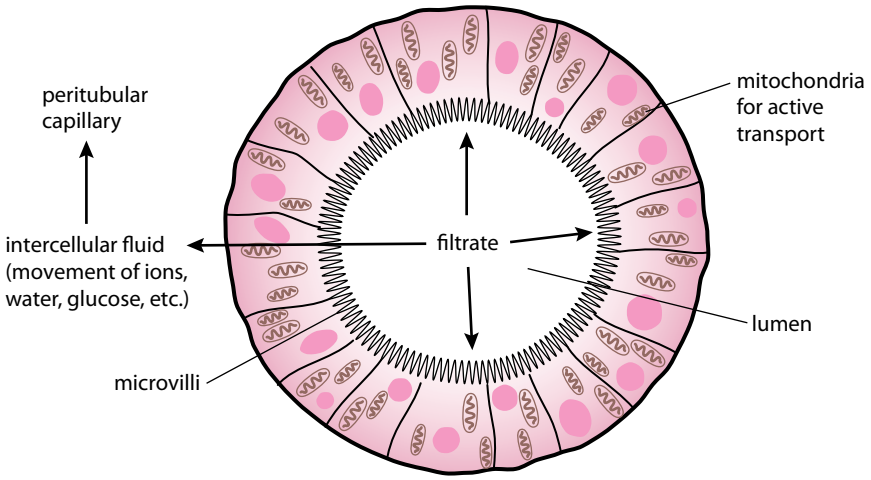


Figure 11.16 Sectioned view of the proximal convoluted tubule of a nephron. Note the adaptations for efficient reabsorption: only one cell layer thick, microvilli present, numerous mitochondria for active transport (ATP), and close to the peritubular capillary bed.

The total volume of your blood is filtered by your kidneys about 25 times each day. This shows how important reabsorption is.

Several transport mechanisms are used in order to accomplish reabsorption. Even though any one type of molecule may be influenced by more than one transport mechanism, there are still some general patterns.

Salt ions

The majority of, although not all, salt ions (e.g. Na^+, Cl^-, K^+) must leave the filtrate and be returned to the bloodstream by reabsorption. The salt ions are first actively transported into the tubule cells and then into the intercellular fluid outside the tubule. Finally, salt ions are taken into the peritubular capillary bed.

Water

The movement of salt ions out of the filtrate and into the tubule cells, intercellular fluid, and peritubular capillary bed, induces water to follow the same route by osmosis. Recall that water moves from a hypotonic region to a hypertonic region following the pathway of the solutes (see Section 1.4). Under normal circumstances much of the water remains in the filtrate awaiting a control mechanism that will determine how much water the body can afford to eliminate in the urine.

Glucose

In a nephron that is functioning properly, all the glucose that is in the glomerular filtrate is reabsorbed into the bloodstream. The only transport mechanism that can explain the totality of this movement is active transport. If glucose was being moved by facilitated diffusion, the highest percentage that could be reabsorbed would be 50% because the concentration gradient disappears once that percentage is reached.

Homeostasis is a term used to describe the body's ability to maintain a stable internal environment. A variety of factors are maintained as part of homeostasis, including blood pH, internal body temperature (birds and mammals), and water balance, to name just a few.

Kidney nephrons and osmoregulation

Water is the solvent of life. It is the solvent in almost all body fluids, including cytoplasm, blood plasma, lymph, and intercellular fluid. Some water needs to be eliminated in the urine each day, but the total volume of water eliminated depends on many physiological factors. These physiological factors include:

- the total volume of water ingested recently, as liquid and in solid foods
- perspiration rate, which is influenced by both exercise level and environmental temperature
- ventilation (breathing) rate, which is largely dependent on the activity/exercise level (a significant amount of water is exhaled when we breathe out).

The body's response mechanisms that attempt to maintain homeostatic levels of water are known as osmoregulation.

Any regulatory mechanism that affects water balance in an animal's body is part of osmoregulation.

The loop of Henle creates a hypertonic environment in the medulla of the kidney

Much of the water in the original filtrate remains after the filtrate has left the proximal convoluted tubule. This water, and the remaining dissolved solutes, enters the descending portion of the loop of Henle. This segment of the loop of Henle is permeable to water but relatively impermeable to salt ions. The filtrate then enters the ascending portion of the loop of Henle, where the tubule is relatively impermeable to water, but permeable to salt ions. As the filtrate moves up the ascending portion of the loop, salt ions are pumped out and enter the intercellular fluid.

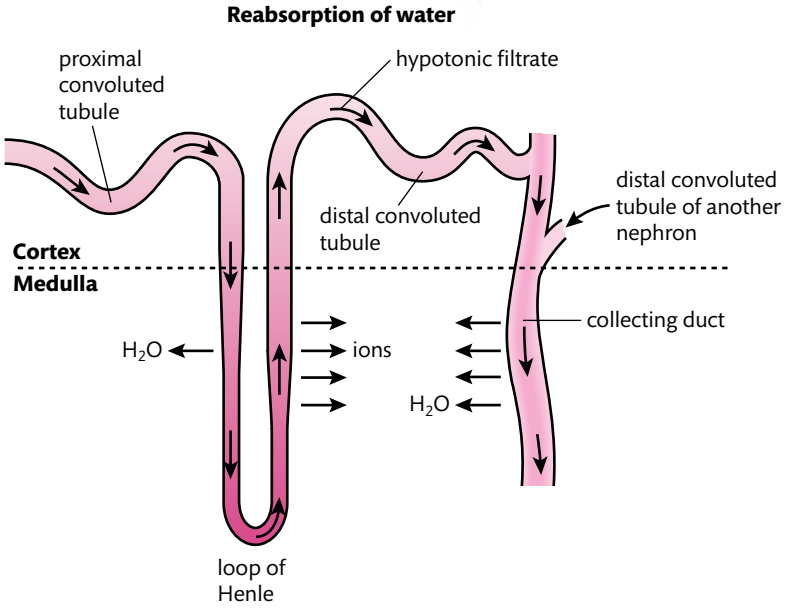


Figure 11.17 Ions move out of the ascending portion of the loop of Henle. This makes the interstitial fluid in the renal medulla hypertonic in relation to the initial urine within the collecting duct.

The loop of Henle of each nephron extends down into the medulla region of the kidney. Thus the medulla is an area with many ions (a hypertonic region) in comparison with the fluids within the tubules or the collecting ducts. Despite the fact that some water moves out of the descending portion of the loop by osmosis, the filtrate that moves up the ascending loop and into the distal convoluted tubule is still relatively hypotonic (has a relatively high water content).

ADH controls reabsorption of water in the collecting duct

The filtrate that enters a collecting duct can be thought of as urine, but urine in a dilute form. The water content is quite high, making this urine hypotonic in relation to the surrounding interstitial fluid of the medulla. If this volume of water was to leave an individual's body consistently as urine, that individual would need an extremely high water intake to make up for this high water loss (not to mention many, many trips to a toilet). Thus, under most circumstances, at least some of this water is reabsorbed through the wall of the collecting duct.

The colour of urine, from relatively colourless to dark yellow, is a clue to how hydrated you are. When urine is nearly colourless, this means that your body has abundant water and is eliminating excess water as dilute urine. After exercising and perspiring a great deal of water, your urine does not contain nearly as much water and the more concentrated solutes give the urine a yellow colour.

The collecting duct is differentially permeable to water. Its permeability depends on the presence or absence of antidiuretic hormone (ADH). ADH is secreted from the posterior lobe of the pituitary gland and, like all hormones, circulates in the bloodstream. The target tissue of ADH is the kidney collecting ducts (see Figure 11.18). Notice that the collecting duct extends into the highly hypertonic interstitial fluid of the medulla. If ADH is present, the collecting duct becomes permeable to water and water moves by osmosis out of the collecting duct and into the medulla interstitial fluid. From there, water enters the peritubular capillary bed and is thus returned to the bloodstream to be made available to all body tissues. If ADH is not present, the collecting duct becomes impermeable to water. Water then stays in the collecting duct, along with the various waste solutes, and the urine is more dilute.

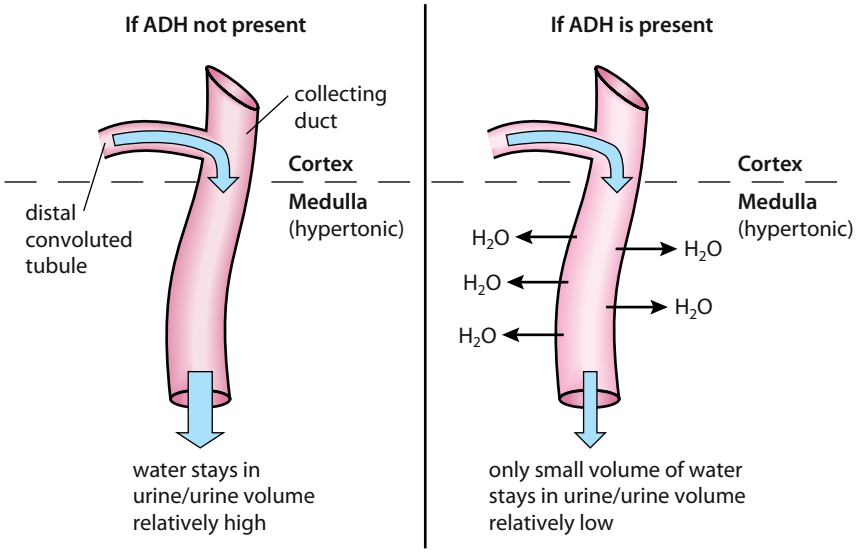


Figure 11.18 Control of water reabsorption in the kidney by ADH.

Try to associate something that you already know to help you learn new knowledge. For example, you may be aware of the expected effect of a diuretic, something you ingest that increases your urine output. If a diuretic increases urine output, an antidiuretic would decrease urine output. Look again at Figure 11.18 and you will see that the name, antidiuretic hormone, makes perfect sense.

A longer loop of Henle is an adaptation for water conservation

Kidneys are highly diverse organs when you compare different species. For example, frogs and toads have virtually no loops of Henle and so are unable to conserve water by the mechanism involving a hypertonic medulla and reabsorption of water from the collecting duct. Their urine is always quite dilute.

At the other end of the spectrum are vertebrate animals that live in desert regions. The scarcity of water means that they have many behavioural and physiological adaptations for water conservation. One interesting and highly studied animal is the kangaroo rat that lives in the desert areas of south-western USA.

The water intake of kangaroo rats comes almost exclusively from the foods that they eat. Kangaroo rats only venture from their burrows during the night, when the air is cooler. They recycle almost all of their water and lose very little of it in the urine they produce. They do this by having a very long loop of Henle that produces a large hypertonic area for water reabsorption using the ADH/collecting duct mechanism.

A banner-tailed kangaroo rat, *Dipodomys spectabilis*.

To learn more about excretion in different animals, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.3.

NATURE OF SCIENCE

You might wonder why desert animals like the kangaroo rat don't solve their water loss problems by producing and excreting uric acid. After all, uric acid production and secretion requires almost no water loss. The answer is simply because kangaroo rats are tied to their evolutionary past. Their ancestors were mammals that lived in less arid regions and had already evolved to use urea as their nitrogenous waste. Desert animals cannot break away entirely from their ancestors, so through the trial and error mechanisms of evolutionary adaptations they have solved their physiological challenges with creative solutions.

What changes do the kidneys make to the blood?

One way to answer this question is to compare the composition of the blood entering a kidney in the renal artery, with the blood leaving a kidney in the renal vein. In a healthy animal the blood leaving in the renal vein, compared with the renal artery, would have:

- a lowered amount of urea
- a lowered amount of salt ions (Na^+, K^+, Cl^-, etc.)
- a lowered amount of water
- a nearly identical amount of glucose
- a nearly identical amount of protein
- absolutely no change in blood cells.

Some animals are osmoregulators and some animals are osmoconformers

In order to achieve consistent water balance, different animals employ different strategies. These strategies are most relevant to water-dwelling animals. The strategies can be classified into two types.

Osmogreulators

Osmoregulators are animals whose internal tissues have a different solute concentration compared with their environment. These animals must have

mechanisms that regulate water balance, and typically expend a great deal of energy in order to achieve those mechanisms. Most animals are osmoregulators.

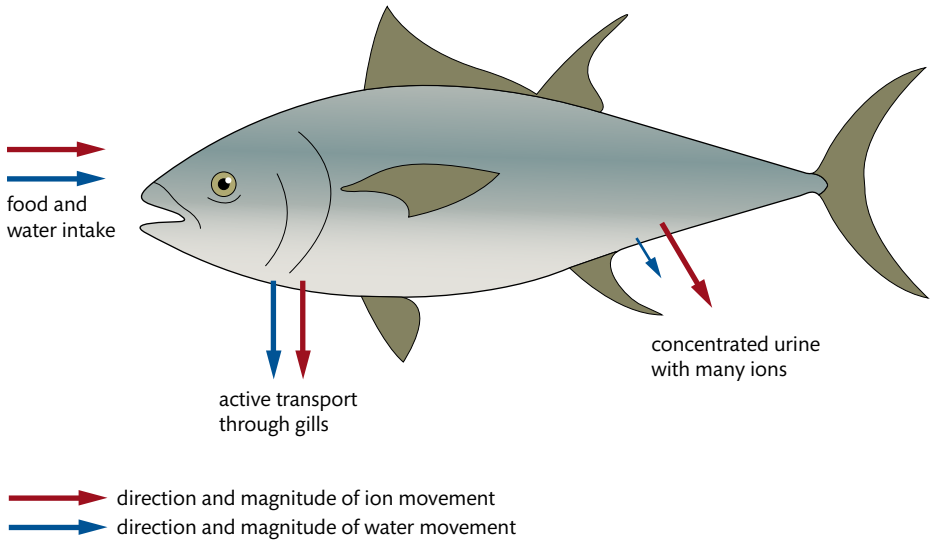


Figure 11.19 Saltwater fish: their tissues are hypotonic in a hypertonic saltwater environment. Notice that saltwater fish expend energy by actively transporting ions out through their gills and in the production of a very concentrated urine.

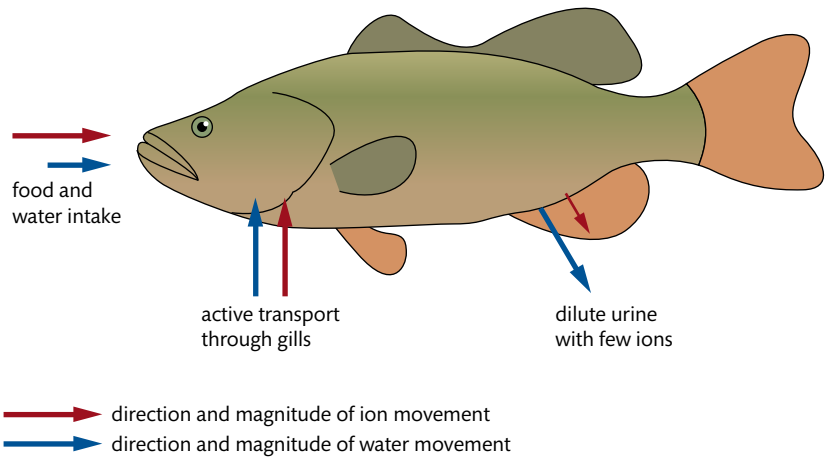


Figure 11.20 Freshwater fish: their tissues are hypertonic to a hypotonic freshwater environment. Notice that freshwater fish expend energy in order to actively transport ions in through their gills and in the production of a very dilute urine.

Osmoconformers

Marine worms and molluscs, such as this bay scallop, are osmoconformers. The solute concentration in their tissues is nearly identical to seawater. An equal volume of water moves into and out of their cells as a result of their iso-osmotic balance with their marine environment.

Osmoconformers are animals that have internal tissues that have virtually the same solute concentration as their environment. They are said to be iso-osmotic to the water of their surroundings. They do not need mechanisms to take in or to eliminate water, as water moves in and out freely because of the osmotic balance. These animals are very restricted to living in only those environments to which they are iso-osmotically matched.

Kidney failure and other medical issues related to kidney function

There are a variety of causes of complete or nearly complete kidney failure. When the kidneys do fail, there are two options available to a patient. One of the options is called kidney dialysis or haemodialysis. During haemodialysis, a patient's blood is pumped into a device that contains a large surface area of a membrane (the dialysis membrane). On one side of the membrane is the patient's blood, and on the other side is a solution (the dialysate) that is similar in chemical makeup to the patient's blood but does not have urea in it at the start of the dialysis. Urea is a small enough molecule to diffuse through the membrane, and so slowly some of the urea leaves the blood and enters the dialysate. The balance of water and some ions can also be regulated by adjusting which fluid on either side of the dialysis membrane has a greater concentration of each substance. Kidney dialysis takes several hours each session and must be repeated every 1–3 days.

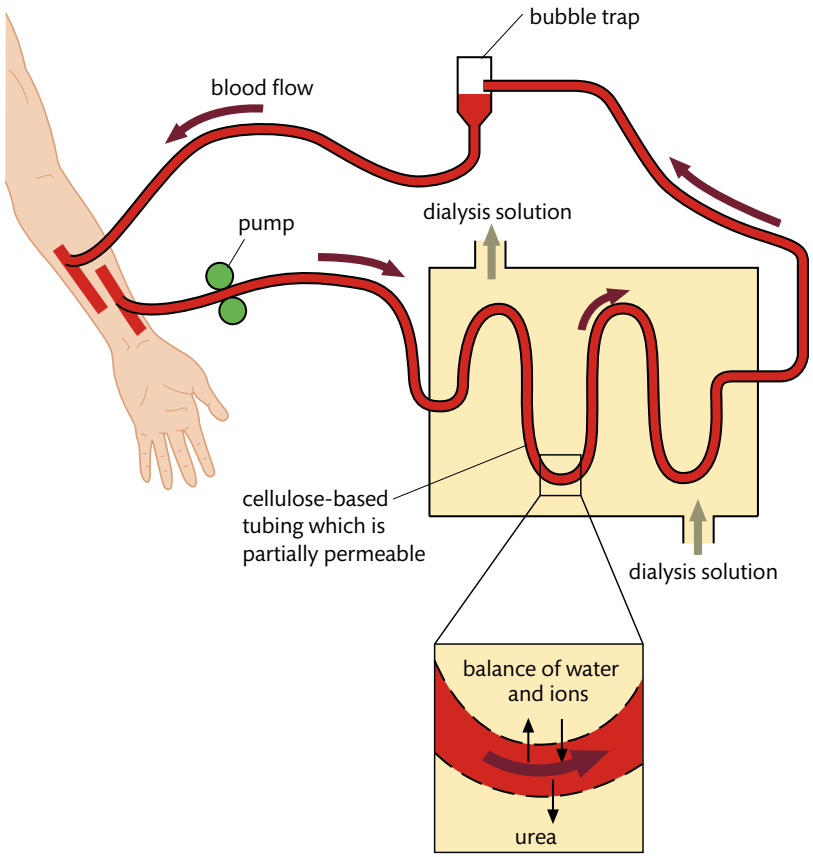


Figure 11.21 Schematic showing how kidney dialysis is performed. The surface area of the dialysis membrane is far greater than shown in this schematic.

A second option when a person's kidneys fail is to have a kidney transplant. Like all transplanted organs and tissues, it is imperative that the patient's and donor's tissues match, in order to minimize rejection of the organ by the patient's immune system. People can live normally with a single kidney, so it is possible for a healthy, close family member to donate a kidney if the blood and tissue type match appropriately. If a family member cannot donate a kidney, the patient will go on a waiting list for a kidney from a deceased donor. Sometimes the waiting list can be quite long. After receiving a transplanted kidney, a patient will need to receive immune-suppressing drugs for the rest of his or her life, as even well-matched kidneys are not a perfect tissue match.

Testing urine for chemical composition

When you visit the doctor, you may have to provide a sample of urine for testing. This is a routine procedure and can provide a doctor with a wealth of information concerning your physiology.

Glucose

In a healthy individual there should be no glucose in the urine. Glucose that is filtered is normally completely reabsorbed in locations such as the proximal convoluted tubule.

Some people develop crystalline structures known as stones within their kidneys that can cause blockages and severe pain, especially if the kidney stones pass into the ureter. Technology has been developed that can often break these stones into small pieces by ultrasound. The small pieces can then pass through the normal urinary tract.

Blood cells

The glomerular filtrate should have no blood cells in it because the blood cells are too large to fit through the fenestrations of the glomerulus. Finding blood cells in urine can be a sign of kidney malfunction or perhaps infection and bleeding somewhere in the renal tubes.

TOK

Modern technology has made it easy for blood and urine tests to be taken, with reasonably accurate and reliable results. Who has the right to have access to the information gained from such tests?

Proteins

Proteins are also too large to make their way through the fenestrations of the glomerulus, and so should not be found in urine.

Drugs

Most drugs make their way into the bloodstream and are filtered by the kidneys. It has become common for some employers (and professional sports teams) to test individuals regularly for unprescribed or unauthorized chemicals.

Dehydration and overhydration

Although unusual for most people, there can be instances when a person drinks more or less water than the body loses by urine output, perspiration, and breathing. This can lead to dehydration (too little water intake) or overhydration (too much water intake). The symptoms for both can be quite serious because water is the solvent component of our blood and cytoplasm. Table 11.3 summarizes some of the symptoms of each.

Table 11.3 Dehydration and overhydration

Dehydration	Overhydration
Sleepiness	Change in behaviour/confusion
Constipation	Blurred vision
Dry mouth and skin	Muscle cramps
Dizziness and headache	Nausea and vomiting

Exercises

9 List all the cellular layers that a molecule would have to pass through in order to be ultrafiltered and then reabsorbed into the bloodstream within a single nephron.

10 Some, but not all, substances can be tested for in the urine of a patient. Why do some substances present in the bloodstream show up in urine samples and others do not?

11 Predict the relative amount of ADH produced by a person who has been drinking lots of water and has not been exercising recently. Justify your prediction.

12 Predict the relative amount of ADH produced by a person who has been exercising vigorously and has not had a chance to hydrate. Justify your prediction.

13 The filtering action of the kidneys does not eliminate urea from the bloodstream. Why is complete elimination of urea not necessary?

14 Are humans osmoregulators or osmoconformers? Justify your answer.

15 Suggest a reason why glucose does show up in the urine of a person who has diabetes and is not currently receiving treatment for the condition?

11.4 Sexual reproduction

Understandings:

- Spermatogenesis and oogenesis both involve mitosis, cell growth, two divisions of meiosis, and differentiation.
- Processes in spermatogenesis and oogenesis result in different numbers of gametes with different amounts of cytoplasm.
- Fertilization in animals can be internal or external.
- Fertilization involves mechanisms that prevent polyspermy.
- Implantation of the blastocyst in the endometrium is essential for the continuation of pregnancy.
- HCG stimulates the ovary to secrete progesterone during early pregnancy.
- The placenta facilitates the exchange of materials between the mother and foetus.
- Oestrogen and progesterone are secreted by the placenta once it has formed.
- Birth is mediated by positive feedback involving oestrogen and oxytocin.

Applications and skills:

- Application: The average 38-week pregnancy in humans can be positioned on a graph showing the correlation between animal size and the development stage of the young at birth for other mammals.
- Skill: Annotation of diagrams of seminiferous tubule and ovary to show the stages of gametogenesis.
- Skill: Annotation of diagrams of mature sperm and egg to indicate functions.

Guidance

- *Fertilization involves the acrosome reaction, fusion of the plasma membrane of the egg and sperm, and the cortical reaction.*

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Assessing risks and benefits with scientific research: the risks to human male fertility were not adequately assessed before steroids related to progesterone and oestrogen were released into the environment as a result of the use of the female contraceptive pill.

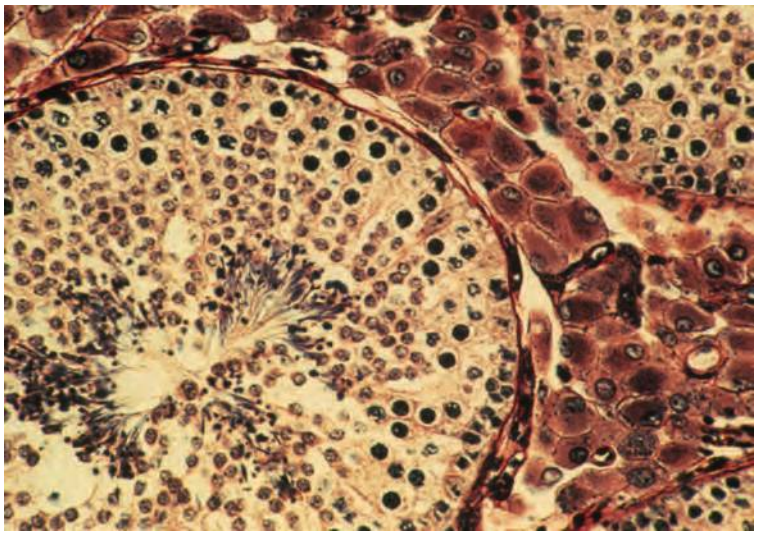
Spermatogenesis: the production of male gametes by meiosis

The production of spermatozoa (sperm cells; singular spermatozoon) occurs within the testes. The testes of human males are located outside the body in order to provide the cooler temperature (lower than the internal body temperature) necessary for production of spermatozoa. Inside each testis, spermatogenesis occurs within very small tubes known as seminiferous tubules. Near the outer wall of the seminiferous tubules lie germinal epithelial cells called spermatogonia (singular spermatogonium). Each spermatogonium is capable of undergoing either mitosis or meiosis at any given time.

Spermatogonia undergo mitosis in order to replenish their numbers. Spermatozoa production starts at puberty and continues throughout life. Millions of spermatozoa can be produced in a single day, and mitosis replaces the cells that become spermatozoa.

Spermatogonia undergo meiosis to produce spermatozoa. Meiosis is also called 'reduction division' because it reduces the original diploid number of chromosomes to the haploid number in gametes. In humans, 23 homologous pairs of chromosomes (46 in total) become 23 individual chromosomes.

When you come across a term like 'germinal epithelium', ask yourself how that term makes sense. In this case, epithelium refers to layers of cells that line hollow organs and glands. Germinal refers to the idea of the earliest stage of development. These two terms perfectly describe spermatogonia. The next time you encounter either of these two terms, you are more likely to understand their context.



A light micrograph of a nearly complete section of a seminiferous tubule. Cells near the outer edge of the tube (shown as the nearly full circle) are the spermatogonia. As the cells progress through meiosis and differentiation, they move closer to the centre. The clear area at the centre is the lumen where many nearly completed spermatozoa with flagella are located. Soon these spermatozoa will detach and move through the lumen to a storage area called the epididymis.

Spermatogonia first replicate the DNA within their still diploid nucleus. At the same time, the spermatogonia are undergoing cell growth in preparation for a cell division. If any one spermatogonium undergoes mitosis, two half-size cells result, each capable of growing again for a later cell division. If a spermatogonium begins meiosis, four spermatozoa are the eventual result.

The steps of meiosis/spermatogenesis

Let's follow the steps of meiosis using human cells as an example. Human spermatogonia are diploid and contain 23 homologous pairs of chromosomes.

A pair of chromatids connected by a centromere is considered to be a single chromosome.

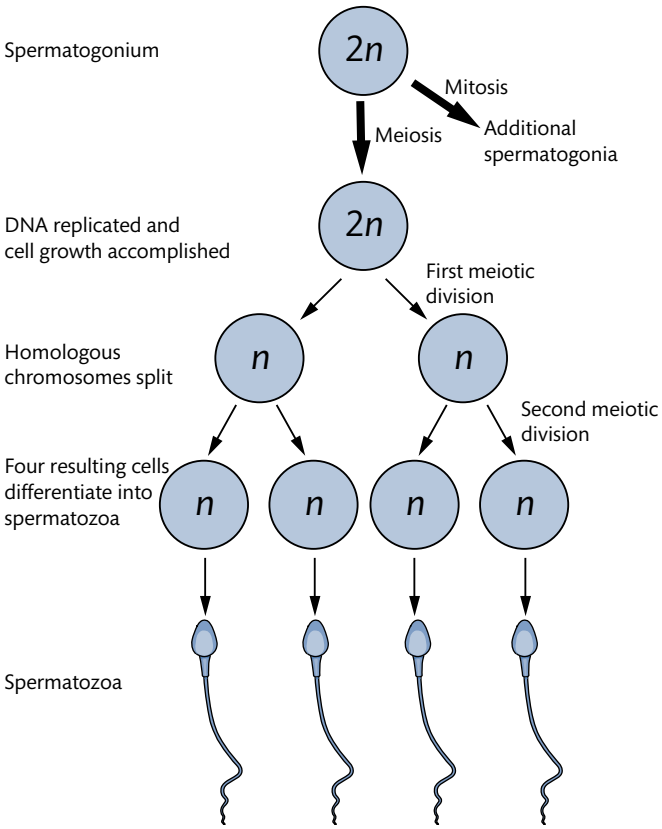


Figure 11.22 The stages of spermatogenesis.

The transition from diploid (2*n*) to haploid (*n*) occurs after meiosis I because this is the cell division that separates the homologous pairs of chromosomes. By definition, diploid cells have homologous pairs of chromosomes and haploid cells do not.

In preparation for meiosis, DNA replication occurs and each of the 46 chromosomes now exists as a pair of chromatids.

Meiosis I occurs and two cells result, each with the haploid number of chromosomes (23) because the homologous pairs have been separated. Each chromosome still exists as a pair of chromatids, so there is another cell division called meiosis II. During meiosis II, the chromatids are separated. Thus four haploid cells,

each containing 23 chromosomes, are created from one that originally contained 23 homologous pairs.

Meiosis is completed for these cells, but each must now differentiate into a fully functioning, motile spermatozoon. Thus the cells remain within the interior of the seminiferous tubule for a period of time as they form the cellular structures characteristic of a mature spermatozoon. These structures include a flagellum for motility and an acrosome that contains the enzymes necessary for fertilization. The developing spermatozoa need nutrients during this period of differentiation, and thus each remains attached to cells in the seminiferous tubules known as Sertoli cells.

Each of the cell stages of meiosis has moved the resulting cell closer to the interior of the seminiferous tubule. Because the tubule is a small tube, there is a cavity or lumen at the centre. Once spermatozoa have completed differentiation, they detach from their Sertoli cell and move through the lumen to the storage area of the testis called the epididymis.

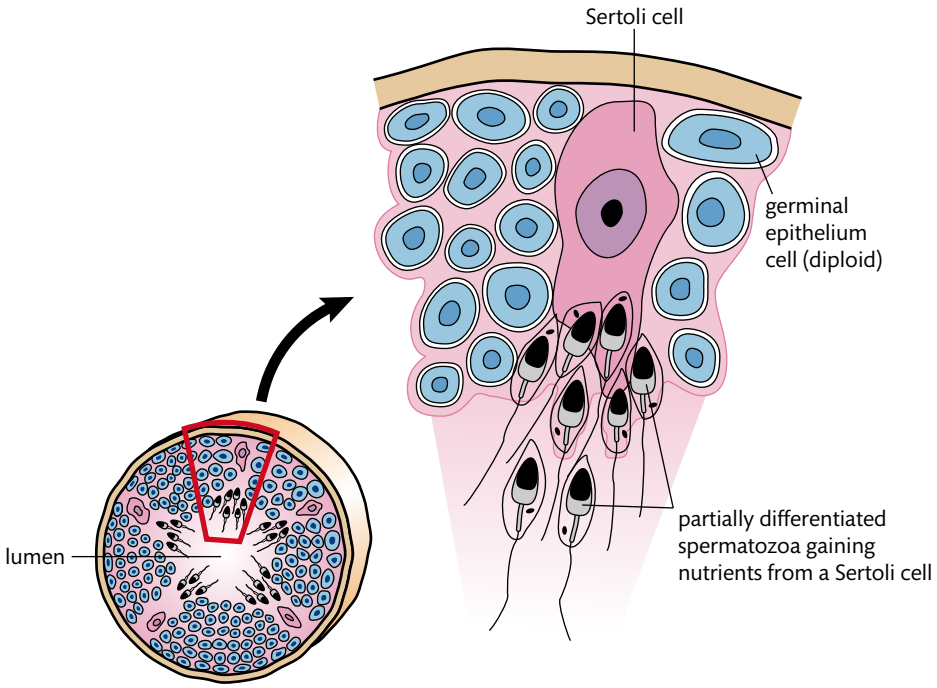


Figure 11.23 A section view through a seminiferous tubule.

Oogenesis: the production of female gametes by meiosis

Oogenesis and spermatogenesis are the female and male processes of meiosis, respectively. Thus there are many similarities between these two processes, especially when the focus is the behaviour of the chromosomes. Oogenesis produces four cells as the 'end products' of meiosis, as does spermatogenesis. However, three of the four end-product cells of oogenesis are not used as gametes because they are much too small to produce a viable zygote if fertilized. These three cells are called polar bodies, and their function is to be a cellular 'container' for the divided chromosomes during both meiosis I and meiosis II. The fourth haploid cell produced is very large and is the ovum (plural ova). As we go through the various developmental stages of an ovum, note the many similarities to spermatogenesis.

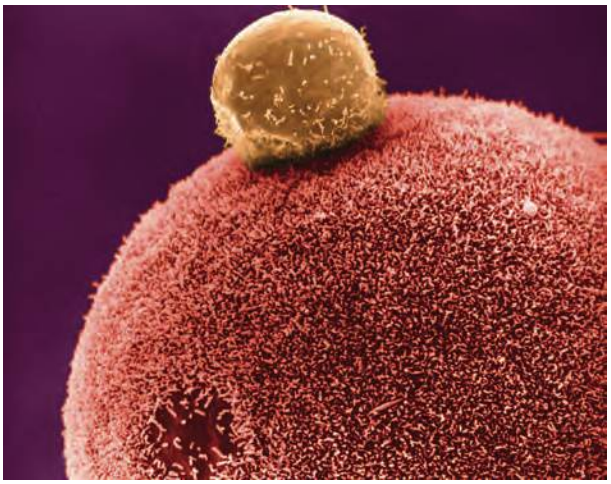
Events occurring before birth

Although many of the events described below are quite similar for other mammals, the details given here are specific to human oogenesis.

Within the ovaries of a female foetus, cells called oogonia undergo mitosis repeatedly in order to build up the numbers of oogonia within the ovaries. These oogonia grow into larger cells called primary oocytes. Both oogonia and primary oocytes are diploid cells. The large primary oocytes begin the early stages of meiosis, but the process stops (is arrested) during prophase I.

Also within the ovaries, cells called follicle cells repeatedly undergo mitosis. A single layer of these follicle cells surrounds each primary oocyte and the entire structure is then called a primary follicle. When a female child is born, her ovaries contain nearly a half million primary follicles. These primary follicles remain relatively unchanged until the girl reaches puberty.

Events occurring with the menstrual cycle



An SEM of a future hamster ovum and one of the polar bodies during oogenesis.

During each menstrual cycle, a few primary follicles finish meiosis I. The two resulting haploid cells are not even close to being equal in size. One is very large and the other is very small. The small cell is called the first polar body and simply acts as a reservoir for half of the chromosomes. The polar bodies produced during oogenesis later degenerate. The other, very large cell is a secondary oocyte.

You will recall that meiosis I produces haploid cells, but each cell has chromosomes existing as paired chromatids. The single ring of follicle cells begins to divide and form a fluid. Two rings of follicle cells are formed, with a fluid-filled cavity separating them. The first (inner) ring of follicle cells surrounds the oocyte, then there is the fluid-filled space, and then the outer ring of follicle cells. This entire structure is now called a Graafian follicle (see Figure 11.24). The increase in fluid between the two follicle cell layers creates a bulge on the surface of the ovary and eventually leads to ovulation.

It is a secondary oocyte with the inner ring of follicle cells that is released from the ovary at ovulation, although people often refer to this event as the release of the ovum or egg. The second meiotic division (meiosis II) is not completed until fertilization. If a fertilization does not occur, the released gamete remains a secondary oocyte until the cell dies. If fertilization does occur, the events stimulate the secondary oocyte to complete meiosis II. Thus there is only a true ovum for the very brief period between the spermatozoa starting to fertilize the female gamete (the secondary oocyte) and the haploid nuclei fusing to form the zygote.

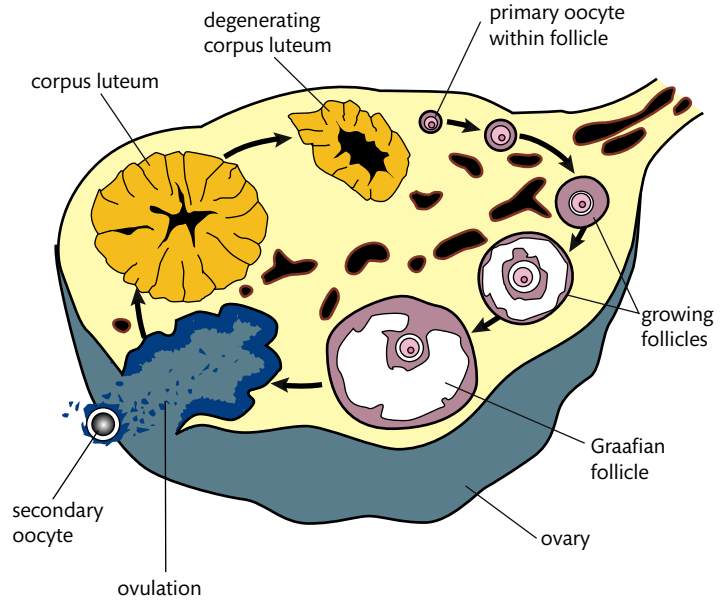


Figure 11.24 An ovary showing the stages in the production of a human Graafian follicle, leading to ovulation and the formation and degeneration of the corpus luteum. This diagram is like a time-lapse photograph of the ovarian events during a single menstrual cycle, as not all of these stages would be occurring at the same time.

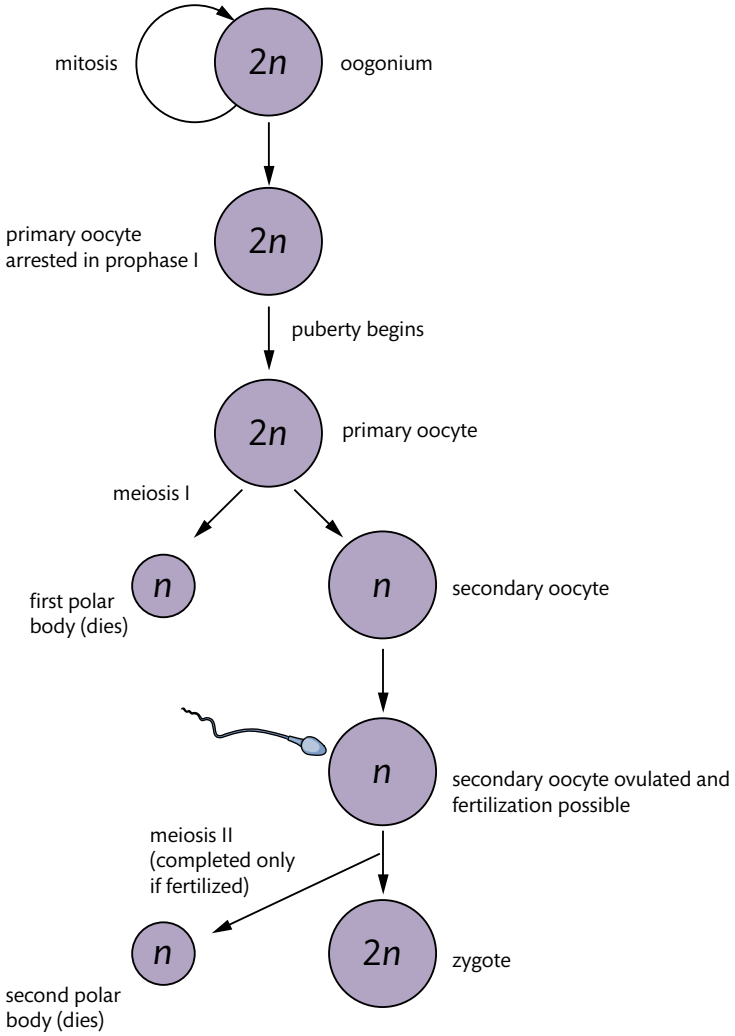


Figure 11.25 Summary of oogenesis. Notice that some of these events occur within the ovary and some occur after ovulation. Oogenesis in humans is a process that takes many years to complete.

The body temperature of most human females increases by about 1°C soon after ovulation. Some couples planning to have children plot the woman's body temperature and time sexual intercourse accordingly. A secondary oocyte is only healthy enough to be fertilized for about a day after ovulation.

Mature spermatozoa and 'ova'

The mature male and female gametes are very well suited for their purpose. Both gametes are haploid. The spermatozoon is a very small cell with a flagellum for motility and mitochondria to provide adenosine triphosphate (ATP) for swimming. At its anterior end, each spermatozoon contains an organelle called an acrosome. The acrosome contains hydrolytic enzymes that help with the fertilization process. Spermatozoa do not contain any unnecessary organelles or structures; their small size allows them to swim great distances and unnecessary structures would be a burden (see Figure 11.26).

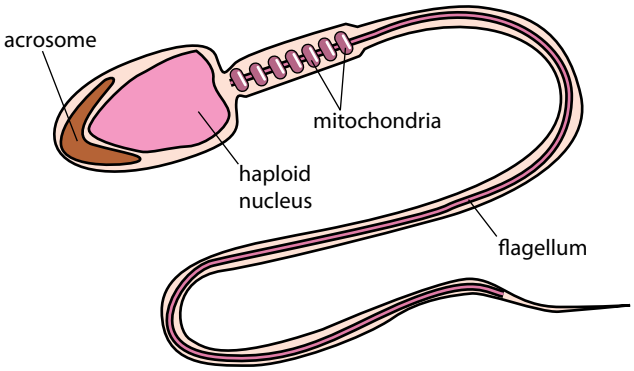


Figure 11.26 A typical mammalian spermatozoa.

By volume, the ovum (secondary oocyte) is the largest cell in most animals. The unequal division of the cytoplasm during meiosis ensures that one cell receives virtually all of the cytoplasm, nutrients, and organelles necessary to start a new life. The nutrients within the ovum are collectively referred to as yolk. In addition, the cytoplasm also contains small vesicles called cortical granules, which function immediately after fertilization. Just outside the plasma membrane is a layer of glycoproteins called the zona pellucida. This also has a function during fertilization.

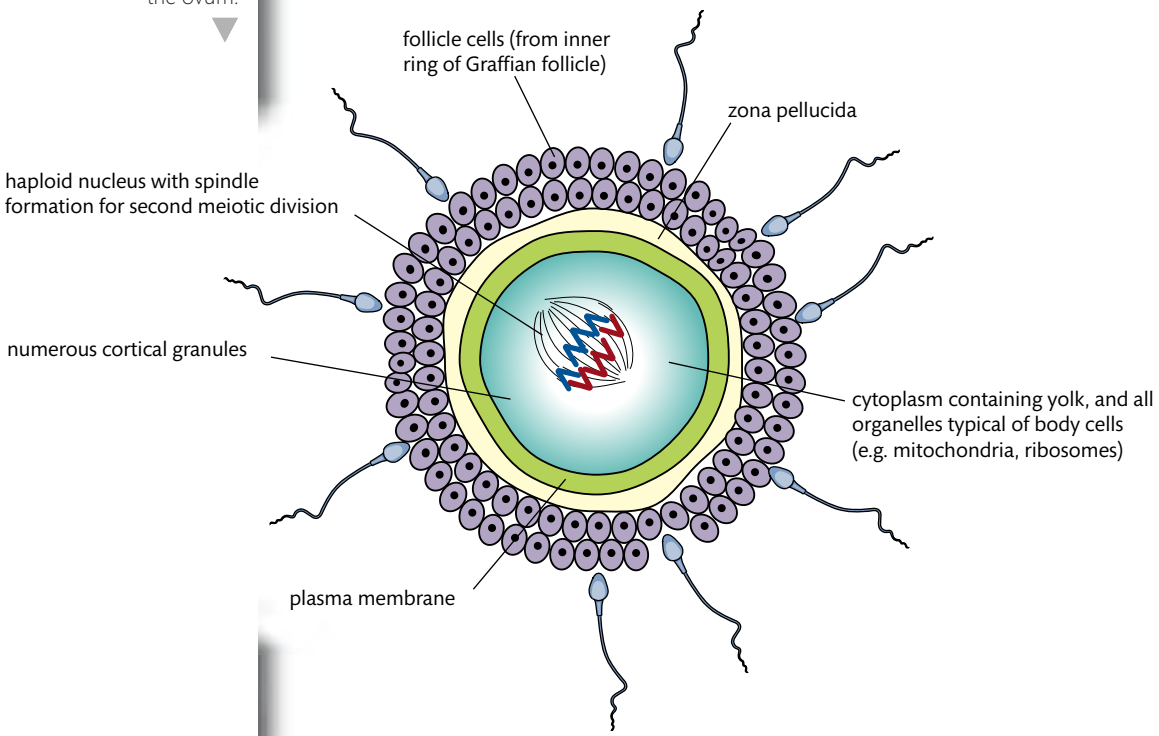


Figure 11.27 A secondary oocyte early in the process of fertilization. Multiple spermatozoa are attempting to fertilize the ovum as meiosis II is being completed within the ovum.

Comparing and contrasting spermatogenesis and oogenesis

The processes by which male and female gametes are produced are easily compared and contrasted, as in Table 11.4.

Table 11.4 Comparing and contrasting spermatogenesis and oogenesis

Spermatogenesis	Oogenesis
Mitosis replaces germinal cells daily	Mitosis replaces germinal cells only early in a female's development
Some cell growth occurs before meiosis I begins	A great deal of cell growth occurs before meiosis I begins
The two divisions of meiosis result in four haploid spermatozoa	The two divisions of meiosis result in one ovum and three possible polar bodies
Spermatids must remain in seminiferous tubules until differentiation into spermatozoon occurs	Differentiation of the oocyte into an ovum occurs partly in the ovary and continues after ovulation
The resulting gamete is extremely small with very little cytoplasm and limited organelles	The resulting gamete is extremely large with a great deal of cytoplasm, nutrients, and numerous organelles
Millions of spermatozoa produced every day throughout life (starting at puberty)	Ovulation of one of a total of thousands of oocytes occurs with each menstrual cycle, then stops at menopause

Fertilization in animals can be external or internal

If a female animal lays eggs to allow the male of the species to fertilize the eggs outside the female's body, the fertilization is said to be external. There are many animals that use this physiological and behavioural strategy in order to reproduce. Good examples are the majority of fish species. A typical female fish often lays hundreds of eggs at a time. A male of the same species swims above where the eggs were deposited and releases a fluid called milt. Milt contains millions of spermatozoa. The system is not very efficient because many of the eggs may never become fertilized, but the vast numbers of eggs laid coupled with the millions of spermatozoa deposited locally typically ensures that a reasonable number of the eggs do become fertilized. Animals that use this mode of fertilization rarely provide parental care for the developing young, and that is another reason for the large numbers of eggs that are laid. Many of the young do not in fact survive predation and other dangers as they mature.

NATURE OF SCIENCE

Contraceptive pills containing relatively high levels of oestrogen and progesterone (sometimes simply referred to as the 'pill') have revolutionized birth control in many areas of the world. The pill fools the female's body into acting as if it was pregnant, by keeping the levels of the two hormones most associated with pregnancy (oestrogen and progesterone) high. Thus the series of events leading to ovulation do not occur when the pill is taken regularly. We also give steroids to other animals. Some cattle farmers use steroids to 'bulk up' their beef cattle. Any organism that takes an outside source of steroids metabolizes the steroid molecules and deposits some of them into its environment in their faeces and urine. In some areas, there is evidence of steroid contamination affecting aquatic ecosystems. In areas of high steroid contamination fish species with lowered egg production and other species of animals with sex determination problems have been documented. This problem was not foreseen, nor was it scientifically studied before steroids had become a common dietary additive for both people and livestock.

CHALLENGE YOURSELF

4 Identify each of these reproductive cells as being haploid or diploid:

- **(a)** a spermatogonium located in the outer perimeter of a seminiferous tubule
- **(b)** a secondary spermatocyte located in a seminiferous tubule
- **(c)** an oogonium located in a foetal ovary of an unborn baby girl
- **(d)** a primary oocyte located in an ovary of a newborn baby girl
- **(e)** a secondary oocyte soon after ovulation in an adult female
- **(f)** a recently fertilized zygote.

To learn more about internal versus external fertilization, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.4.

A second reproductive strategy employed by animals is internal fertilization. The female and male engage in some form of intercourse in which spermatozoa are deposited into the female in order to fertilize one or more ovum. The number of ova produced by females for internal fertilization is typically far less than the number of ova laid by species that use external fertilization. Some animals that use internal fertilization also display a high level of parental care of the young. These animals only need to produce a small number of ova because the reproductive success for these animals is quite high. An excellent example is the American alligator. Fertilization is internal and the eggs are laid in a nest area that the female creates. She then guards the developing embryos closely as they incubate. For a period of time after hatching, she also protects the young from predators, including any male alligators in the area, who appear to show no paternal instincts towards the young. Much of the aggressive behaviour attributed to alligators arises when people disturb a female who is protecting a nest or young hatchlings.

An American alligator, *Alligator mississippiensis*, with one of her young. Alligator females are very protective of their young.

Fertilization

Many of the events associated with fertilization depend on the species of animal concerned and factors such as whether the fertilization is external or internal. What will be described here are the events that occur before, during, and after fertilization in humans.

As a result of sexual intercourse, millions of spermatozoa are ejaculated into a female's vagina. The motile spermatozoa absorb some of the fructose sugar in semen in order to have 'fuel' for what could be a very long journey. At least some of the spermatozoa find their way through the cervical opening (the cervix separates the vagina and the uterus) and gain access to the uterus. They begin swimming up the endometrial lining, and some enter the openings of the two Fallopian tubes. If the female is near the middle of her menstrual cycle, there may be a secondary oocyte within one of the two Fallopian tubes. The reason for millions of spermatozoa in each ejaculate becomes clear when you consider that only a very small percentage of the motile spermatozoa will ever reach the location of the secondary oocyte.

Reproductive biology and birth control issues are important from an international perspective as the world's human population continues to grow. At the time of writing, the Earth's population is estimated to be about 7 billion people and still growing. The countries with the top three population sizes are China, India, and USA.

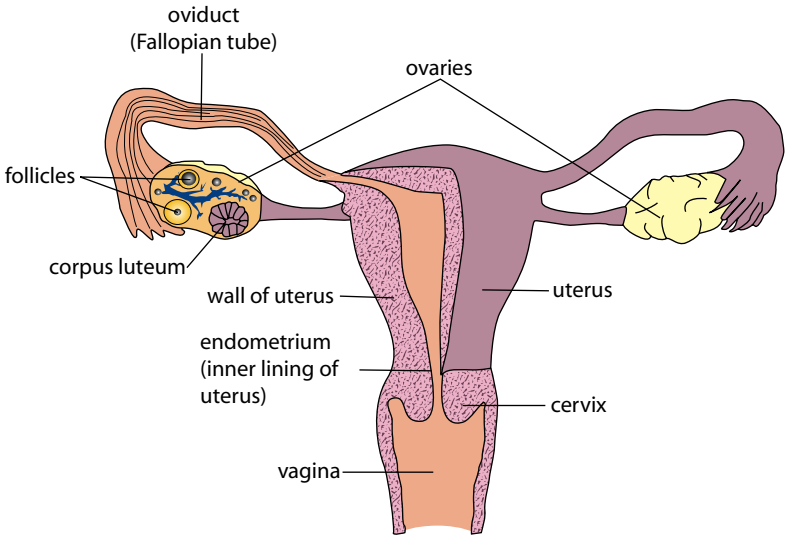


Figure 11.28 A secondary oocyte is ovulated from one of the two ovaries and enters the corresponding Fallopian tube. Spermatozoa ejaculated into the vagina swim up through the cervix, then the inner lining of the uterus, and enter the Fallopian tubes. Fertilization usually occurs within the Fallopian tube. The resulting embryo begins developing immediately as it continues its journey towards the uterus.

There is about a 1 in 250 million chance that any single spermatozoon ejaculated in the vagina will be the one that will fertilize the secondary oocyte. You would not be 'you' if you had not won that lottery long ago.

The typical location for fertilization is within one of the Fallopian tubes. No single spermatozoon can accomplish the entire act of fertilization because it takes many spermatozoa to penetrate the follicle cell layer and a coating called the zona pellucida surrounding the secondary oocyte (see Figure 11.27). Several spermatozoa gain access to the zona pellucida (a glycoprotein gel layer) surrounding the secondary oocyte and release the hydrolytic enzymes contained in their acrosomes, an event simply called the acrosome reaction. One spermatozoon will reach the plasma membrane of the secondary oocyte first, and will use the hydrolytic enzymes of its acrosome to penetrate the egg. The plasma membranes of the two gametes fuse together. This spermatozoon then donates its paternal set of haploid chromosomes to the maternal set already contained in the ovum.

More than one spermatozoon fertilizing an ovum is called polyspermy and results in multiple sets of chromosomes within the ovum. Polyspermy will not lead to a viable embryo. To prevent this, the first spermatozoon penetrating the plasma membrane of the ovum initiates a series of events called the cortical reaction. The cortical reaction is designed to prevent more than one spermatozoon from fertilizing the ovum. Within the cytoplasm of the ovum are many small vesicles called cortical granules; they are located all around the interior of the plasma membrane. When the first spermatozoon and ovum fuse their plasma membranes,

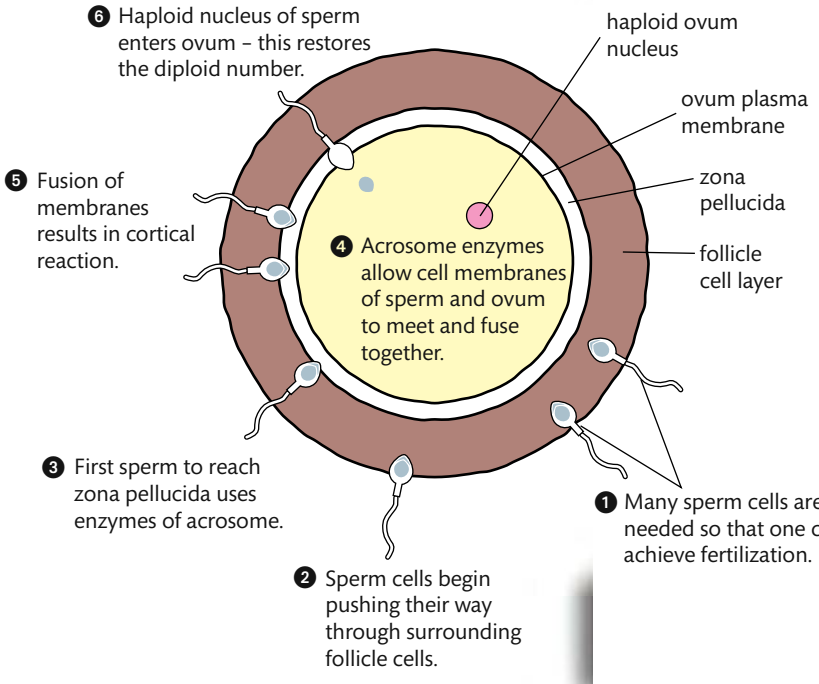


Figure 11.29 The sequence of events in mammalian embryos preventing polyspermy.

To learn more about polyspermy, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.4.

the cortical granules fuse with the ovum's internal plasma membrane and release their enzymes to the outside. These enzymes result in a chemical change in the zona pellucida, making it impermeable to any more spermatozoa. The cortical reaction takes place within a few seconds of the first spermatozoon gaining access and ensures that only one spermatozoon actually fertilizes the ovum. The resulting fertilized ovum is now referred to as a zygote. The diploid condition ($2n$) has been restored and a new life has been started.

Many animals prevent polyspermy by reversing an electrical (ion) charge upon first fertilization. The technique has been well studied in sea urchins. Sea urchin ova start by having a negative electric charge on the inside of their plasma membrane. When the first spermatozoon fertilizes an ovum, the charge is immediately reversed to positive. Further spermatozoa are repelled by the positive charge.

TOK

Many private clinics have opened in the last couple of decades that offer medical assistance for reproduction. One of the services commonly offered by such clinics is IVF: *in vitro* fertilization. It is standard practice to screen embryos in order to select only healthy embryos to implant into the mother. A question that society will eventually have to answer is what constitutes an acceptable level of screening? Is predicted intelligence acceptable screening? Is predicted athletic ability acceptable screening? Science doesn't provide answers to questions like these.

Early development: implantation into the endometrium by the blastocyst

Fertilization stimulates the zygote to begin a mitotic division, and the first division typically occurs approximately 24 hours after fertilization. The rate of mitotic division will increase with subsequent divisions. The early embryo continues to move within the Fallopian tube towards the cavity of the uterus as it divides. The rate of mitotic division continues to increase and, by the time the embryo reaches the uterine cavity, it is approximately 100 cells in size and is ready to implant itself into the endometrium of the uterus. The embryo at this stage is a hollow ball of cells and is called a blastocyst (see Figure 11.30). A blastocyst is characterized by:

- a surrounding layer of cells called the trophoblast, which will help form the foetal portion of the placenta
- a group of cells on the interior known as the inner cell mass, located towards one end of the blastocyst; the inner cell wall mass will become the body of the embryo
- a fluid-filled cavity.

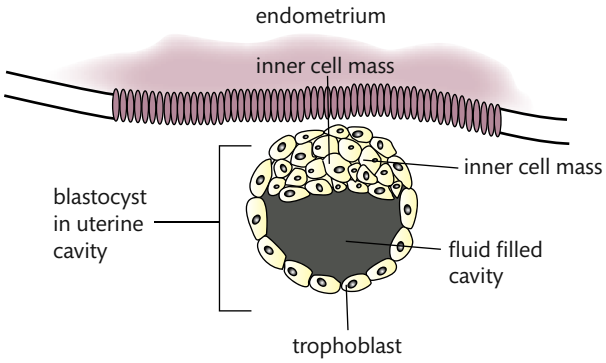
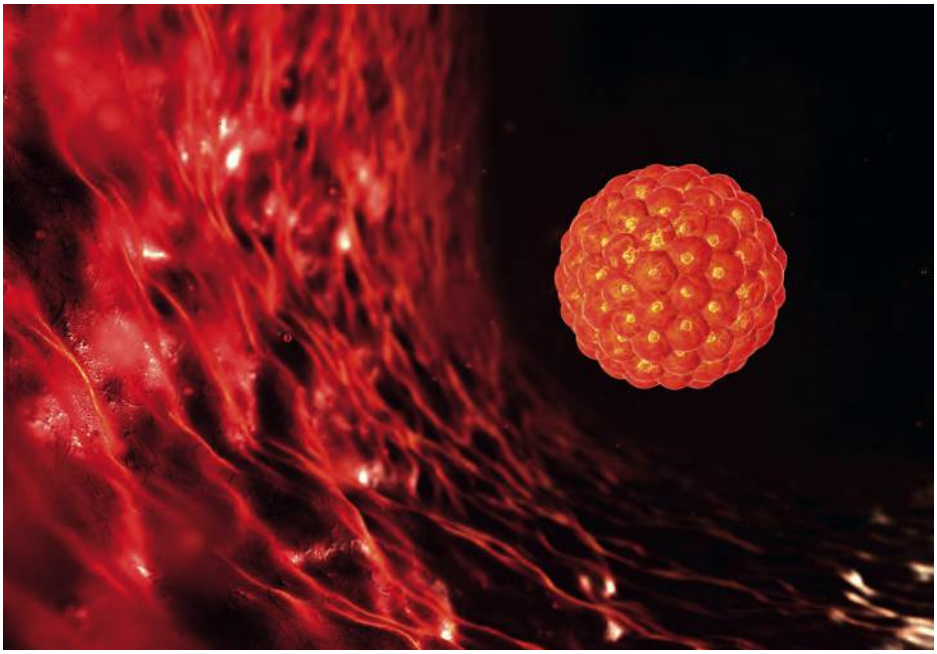


Figure 11.30 A human blastocyst shown in section. This blastocyst has reached the endometrium of the uterus and is going to implant itself through the cell layers shown in colour.

When the blastocyst enters the inner cavity of the uterus, it is in direct contact with the inner lining called the endometrium. The timing of the menstrual cycle, including ovulation, ensures that the endometrium is highly vascular (has many small blood vessels, including capillary beds) at this point in time. The embryo will eventually stop moving along the endometrium and begin a series of steps that allows it to sink down into the endometrial tissue. This is why this step of pregnancy is called implantation. The primary reason that a human ovum is so large is because it contains the nutrients needed for early embryonic development. During the first week after fertilization, there is no true growth of the embryo. The cell divisions that occur create an embryo of 100 or more cells, but the overall size of the embryo is no larger than that of the original ovum. The nutrients stored within the ovum have been used for metabolism, not for growth. When the human embryo begins implantation into the wall of the endometrium, it is rapidly running out of stored nutrients (yolk). Fortunately, as a result of implantation, the embryo and the maternal endometrium soon begin to create a structure known as the placenta.



Computer artwork showing a blastocyst (right) preparing to implant itself into the endometrium of the uterus (left). Notice that, even though the blastocyst is a hollow ball of cells containing an inner cell mass, from the outside it simply looks like a ball of cells.

Role of the placenta

The placenta forms from the trophoblast layer of the blastocyst (see Figure 11.30). The placenta forms from tissue from both the embryo and the mother. Think of the placenta as being a large pancake-shaped structure. The side of this 'pancake' that is further into the uterine wall is made of connective tissue and blood vessels formed by the mother. The side closer to the embryo is formed by the embryo and also contains connective tissue and small blood vessels. On the foetal side of the placenta a protective sheath called the umbilical cord will develop that covers three foetal blood vessels. When fully formed, two foetal blood vessels within the umbilical cord carry foetal blood to the placenta. The blood within these two vessels is deoxygenated and carries waste products. This foetal blood exchanges materials with the maternal bloodstream, and the third foetal blood vessel returns the blood to the foetus. The blood that returns to the foetus has been oxygenated, and nutrients have been added while it passes through the placenta.

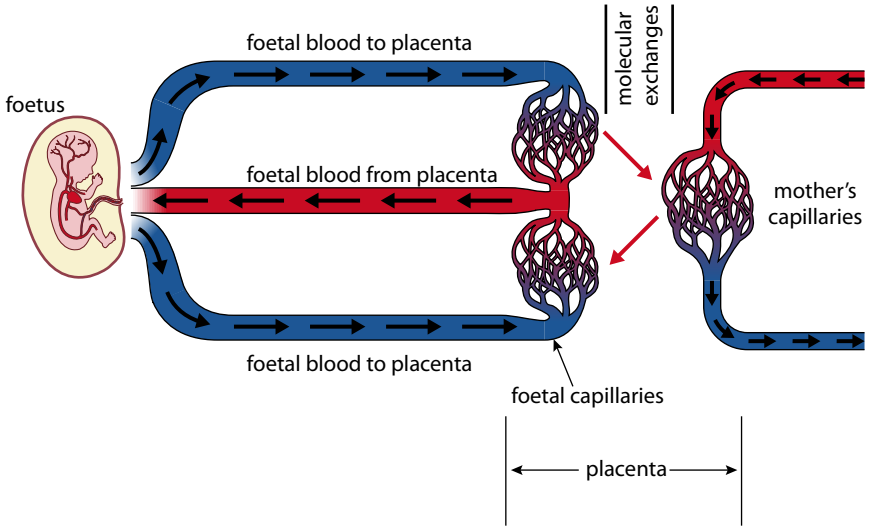


Figure 11.31 A schematic showing the blood flow pattern of the placenta. The three foetal blood vessels shown are contained within the umbilical cord. No blood is ever exchanged between the mother and foetus because their blood vessels do not join.

The molecular exchanges that typify the placenta are shown in Table 11.5.

Table 11.5 The molecular exchanges of the placenta

Molecules passed from foetus to mother within the placenta	Materials passed from mother to foetus within the placenta
Carbon dioxide	Oxygen
Urea	Nutrients (glucose, amino acids, etc.)
Water	Water
Hormones	Hormones
	Vitamins and minerals
	Alcohol, nicotine, and other drugs if used by the mother during pregnancy

At no time does the blood of the foetus and the blood of the mother actually mix: there is an exchange of molecules, but no exchange of blood.

The early embryo and placenta secrete hormones

When a female becomes pregnant, the early embryo secretes a hormone called human chorionic gonadotropin, abbreviated to HCG. This is the hormone that early pregnancy tests (EPTs) are designed to detect. The function of HCG is to enter the mother's bloodstream and 'maintain' the corpus luteum of her ovary for a longer period of time compared with a typical menstrual cycle. The function of the corpus luteum is to secrete progesterone to maintain the highly vascular endometrium of the uterus. By prolonging the existence of the corpus luteum, the endometrium does not degenerate, so the embryo can implant itself into the rich vascular tissue.

Eventually the placenta itself takes over the production of progesterone during the pregnancy, and continually increases the amount of progesterone during the entire

To learn more about placental hormones, go to the hotlinks site, search for the title or ISBN, and click on Chapter 11: Section 11.4.

gestation. In addition, the placenta begins production of high levels of oestrogen that also continually increase during the entire gestation.

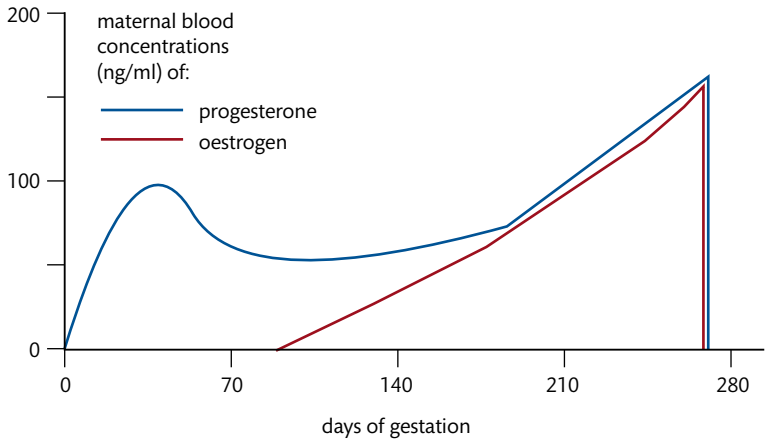


Figure 11.32 A graph showing the levels of progesterone and oestrogen in a pregnant human female during the entire gestation. The very early rise in progesterone is secreted by the ovary (*corpus luteum*), but the source of most of the progesterone is the placenta. The placenta does not produce measurable levels of oestrogen until the final two-thirds of the pregnancy. The sudden plummet of both hormones is simply the result of the birth because the source of the two hormones is no longer present.

Table 11.6 Some of the functions of progesterone and oestrogen during pregnancy

Progesterone	Oestrogen
Helps maintain the highly vascular tissue characteristic of the uterus/placenta	Encourages muscle growth of the uterus
Suppresses contractions of the smooth muscle of the uterus (the uterus is highly muscular, for the birth contractions)	Eventually 'antagonizes' the action of progesterone to suppress uterine contractions
	Stimulates mammary gland development late in pregnancy in preparation for milk production
	Induces production of oxytocin receptors in uterine muscles late in pregnancy (see below for the function of oxytocin)

Both progesterone and oestrogen inhibit the production of any further oocyte development during the entire pregnancy. This explains why a human female cannot become pregnant while she is already pregnant.

The hormonal events associated with birth

Most feedback mechanisms in physiology are designed to work by negative feedback. This works well when there is a physiological factor such as body temperature or blood glucose that needs to be maintained within a fairly narrow homeostatic range. Birth, or parturition, is a process that is not a normal part of mammalian homeostasis. Parturition is characterized by uterine contractions that begin at a relatively low intensity and occur infrequently. As birth continues, the uterine contractions become more and more intense, and become more and more frequent. The feedback control that is involved is called positive feedback. In effect, a previous event results in a more forceful and frequent future event. There is no homeostatic factor being controlled, the series of events will only culminate when birth occurs.

A giraffe giving birth. Placental mammals are the most successful mammals. The events involving birth in other placental mammals have some differences compared with humans, but there are many similarities as well.

Pregnant women that are past term are sometimes given a chemical called pitocin to induce labour. Pitocin is recognized by receptors of the uterus as oxytocin. This begins the positive feedback loop leading to birth.

The hormone that is primarily involved in this positive feedback mechanism is oxytocin. As shown in Table 11.6, one of the effects of the oestrogen produced by the placenta is to induce production of oxytocin protein receptors in uterine muscle. Oxytocin is a hormone produced by the hypothalamus and secreted by the posterior pituitary gland. When the time for birth has come, the posterior pituitary will release a small amount of oxytocin into the bloodstream. Oxytocin receptors in the muscle of the uterus will respond with the first contraction. That first contraction will signal the hypothalamus to signal the posterior pituitary to release slightly more oxytocin. This happens over and over again until the uterine contractions are very intense and very frequent. The event that will terminate the positive feedback loop is birth because the uterine muscle will no longer have anything to contract upon.

Exercises

16 Briefly outline how a spermatozoon is well adapted for its function.

17 Briefly outline how an ovum is well adapted for its function.

18 What is the fundamental difference between the feedback mechanism involving oxytocin and birth and the mechanism involving insulin and blood sugar?

19 What are the primary disadvantages of external fertilization?

20 Why can a mother and her foetus have different blood types, yet there is no immune response if the blood types are incompatible?

Practice questions

1 Explain the role of antibody production with regard to vaccinations.

(*Total 8 marks*)

2 **(a)** Draw the structure of a mature human egg. (4)

(b) Compare the processes of mitosis and meiosis. (6)

(c) Explain how both meiosis and fertilization promote variation in a species that leads to natural selection. (8)

(*Total 18 marks*)

3 Explain how skeletal muscle contracts.

(*Total 9 marks*)

4 Describe the roles of structures at the elbow joint, including nerves, muscles and bones, in movements of the human forearm.

(*Total 8 marks*)

5 When a nerve impulse is received by skeletal muscle it initiates a number of processes that result in contraction. What are the roles of Ca^{2+}, tropomyosin and ATP in contraction?

	Ca^{2+}	Tropomyosin	ATP
A	binds to troponin	exposes binding site	binds to myosin
B	binds to myosin	exposes binding site	binds to troponin
C	binds to actin	exposes binding site	binds to myosin
D	binds to troponin	binds to myosin	binds to actin

(*Total 1 mark*)

6 What is the role of ligaments in the elbow joint?

A Attach biceps to radius

B Reduce friction between humerus, ulna and radius

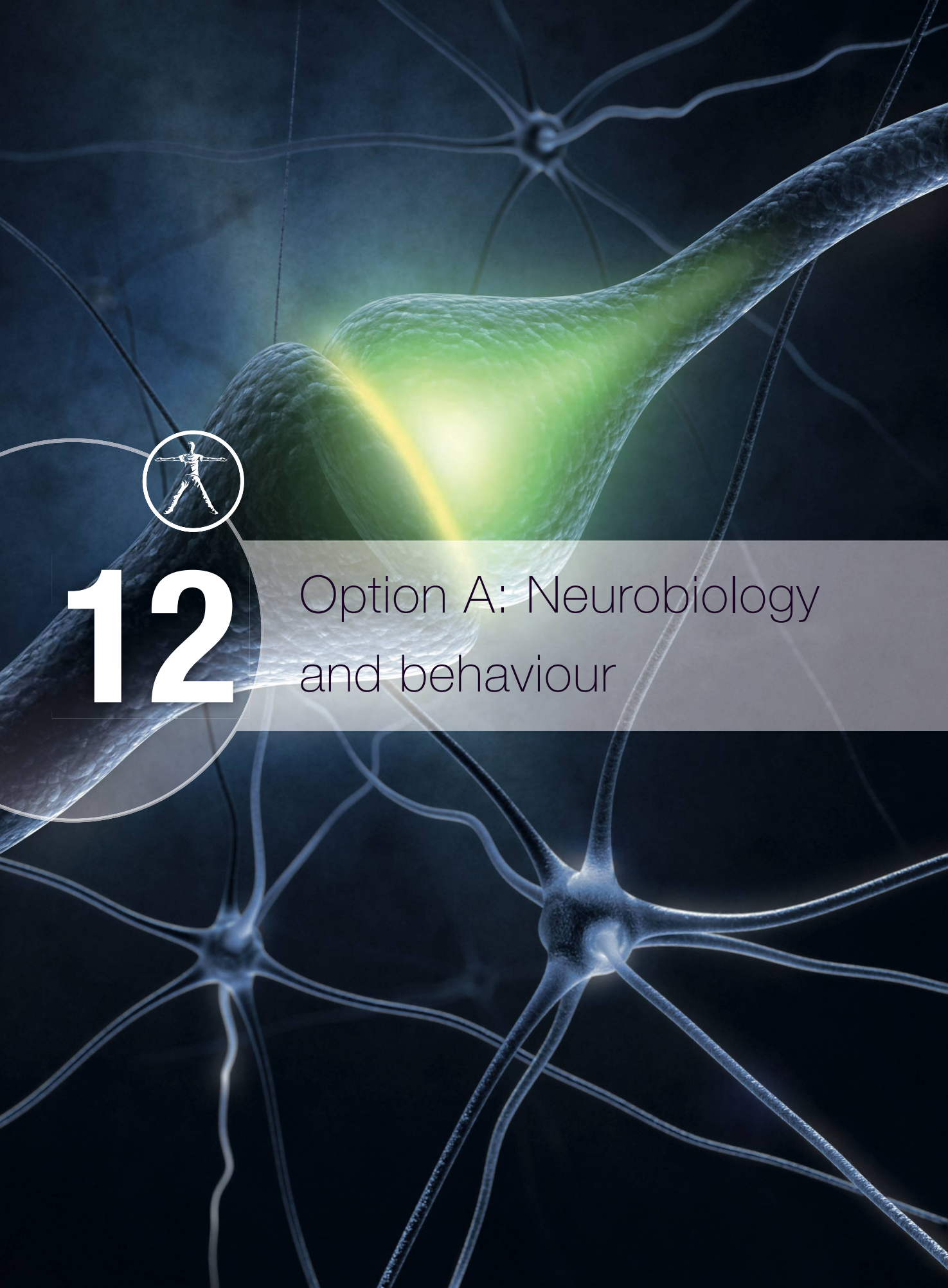
C Hold humerus, ulna and radius in proper alignment

D Secrete synovial fluid

(*Total 1 mark*)

7 Explain the control of ADH (antidiuretic hormone) secretion.

(*Total 6 marks*)



12 Option A: Neurobiology and behaviour

Active neurones in the brain are communicating using chemical messaging.

Essential ideas

A.1	Modification of neurones starts in the earliest stages of embryogenesis and continues to the final years of life.
A.2	The parts of the brain specialize in different functions.
A.3	Living organisms are able to detect changes in the environment.
A.4	Behavioural patterns can be inherited or learned.
A.5	Communications between neurones can be altered through the manipulation of the release and reception of chemical messengers.
A.6	Natural selection favours specific types of behaviour.

Do video games have a long-term effect on brain functioning? Does learning a second language make your brain more efficient? Scientists, using both animal models and new technologies, are now discovering the answers to these questions. For example, Yang Wang, a radiologist from the School of Medicine in Indiana, is using functional magnetic resonance imaging (fMRI) to study the brains of young adults watching violent video games. Technology has also given us the ability to collect data on how neurones migrate in the developing brain and communicate with each other. We now know that the brain is plastic throughout our lives. It keeps on being moulded through new experiences, like learning a new language. Research on how drugs affect the brain has allowed medications to be developed that improve the lives of people with biochemical imbalances. Animal models have helped us understand the problem of addiction.

The study of neurogenesis in the embryonic brain has provided us with data showing that neurones are producing and responding to chemical messages. Nerve cells communicate with each other using molecules. As the immature nerve cells migrate to their final home and the brain matures, millions of connections are formed and then lost. Those connections that are reinforced by experience remain as learning and memory.

Animal behaviour is another fascinating area of research. Why do vampire bats share blood with each other, and why do baby ducks follow their mothers around? All of these questions are addressed with data collected from both behavioural and genetic studies. The answers help us learn how these animals survive to reproduce, but also to understand more about how we interact with our natural world.

NATURE OF SCIENCE

Use models as representations of the real world: developmental neuroscience uses a variety of animal models.

A.1 Neural development

Understandings:

- The neural tube of embryonic chordates is formed by infolding of ectoderm followed by elongation of the tube.
- Neurones are initially produced by differentiation in the neural tube.
- Immature neurones migrate to a final location.
- An axon grows from each immature neurone in response to chemical stimuli.
- Some axons extend beyond the neural tube to reach other parts of the body.
- A developing neurone forms multiple synapses.
- Synapses that are not used do not persist.
- Neural pruning involves the loss of unused neurones.
- The plasticity of the nervous system allows it to change with experience.

Applications and skills:

- Application: Incomplete closure of the embryonic neural tube can cause spina bifida.
- Application: Events such as strokes may promote reorganization of brain function.
- Skill: Annotation of a diagram of embryonic tissues in *Xenopus*, used as an animal model, during neurulation.

Guidance

- *Terminology relating to embryonic brain areas or nervous system division is not required.*

Neural tube formation

Have you ever wondered how all the organs in our body form from just one fertilized egg? The study of this development, from a fertilized egg to a fully formed organism, is called embryogenesis. Scientists have come to understand the processes of embryogenesis by studying various animal models. Because the ultimate goal is to understand embryogenesis in humans, animals in the same phylum with similar developmental patterns have been studied. Humans belong to the phylum Chordata (chordates) and are in the subphylum Vertebrata (vertebrates). Vertebrates, which include fish, amphibians, reptiles, birds, and mammals, are all therefore considered as possible animal models. A frog is an animal that has been studied extensively because it is (or was) readily available and can be collected from local ponds by scientists. During the earliest part of the 20th century, chicks were added to the study of embryogenesis. Birds are a warm-blooded vertebrate, and fertile chick eggs are available all over the world for scientists to use, with very little expense involved. Historically, scientists moved away from the study of 'lower' chordates such as frogs to the study of 'higher' chordates such as chicks, with the aim of understanding normal and abnormal embryogenesis.

One of the benefits of these studies of embryogenesis is that it enabled scientists to learn the key principles of neural development. Using the frog embryo as an example, we can see how the nervous system of an embryonic chordate develops.

After fertilization, cells of the frog embryo develop into three distinct tissue layers: the outermost layer (ectoderm), which will become the brain and nervous system of the adult frog; the inner layer (endoderm), which forms the lining of the gut and the lining of other organs; and the middle layer (mesoderm), which develops into the skeletal, reproductive, circulatory, excretory, and muscular system of the adult frog. A cavity in the centre of the frog (*Xenopus*) embryo is a primitive gut called the archenteron.

From these layers, one of the first organs to develop is the neural tube, which will eventually become the brain and spinal cord of the frog. In embryos, the presence of one tissue that is developing causes the development of another tissue. In this case, the presence of the notochord, a mesodermal tissue, causes the ectoderm to develop into a neural plate. As embryogenesis continues, the neural plate folds in, closes, and becomes the neural tube. The neural tube then elongates and becomes the brain and spinal cord of the frog.

Here is a picture of the embryonic tissues of *Xenopus* (a species of frog).

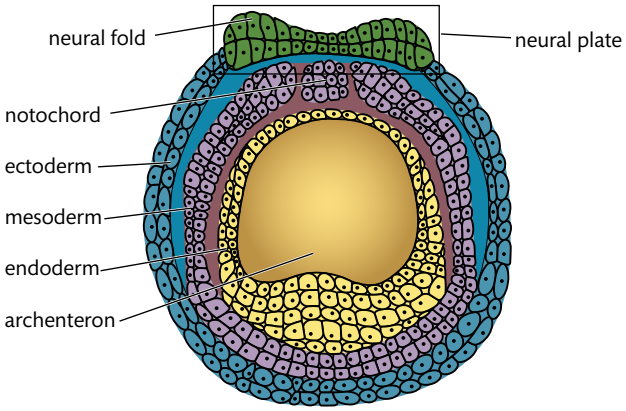


Figure 12.1 Embryonic tissues in *Xenopus*. Campbell and Reece 1999

CHALLENGE YOURSELF

1 Redraw the picture of the embryonic tissues of *Xenopus* and label it without looking at Figure 12.1. Annotate the figure you have drawn. To annotate you must describe the fate of each part as it develops into the adult *Xenopus* frog. Annotate using a table of your own design.

NATURE OF SCIENCE

What are model organisms? Model organisms are organisms that are easy to study, and are used widely by scientists studying in a similar field. The following table will give you an idea of what organisms provide good models for particular studies, and why.

Field of study	Characteristics needed for the model/why the species provides a good model	Suitable species
Genetics	Large numbers and short generation times	Fruit fly Baker's yeast Nematode worm
Developmental biology	Robust embryos that are easily manipulated	Chicken African clawed frog (*Xenopus*)
Genomic studies, such as genes that cause diseases	60% of human genetic diseases studied have a counterpart in the fruit fly and nematode	Fruit fly Nematode
Comparative genomics	The mouse genome is similarly organized to the human genome	Mouse

Table 12.1 Examples of model organisms

To learn more about model organisms, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.1.

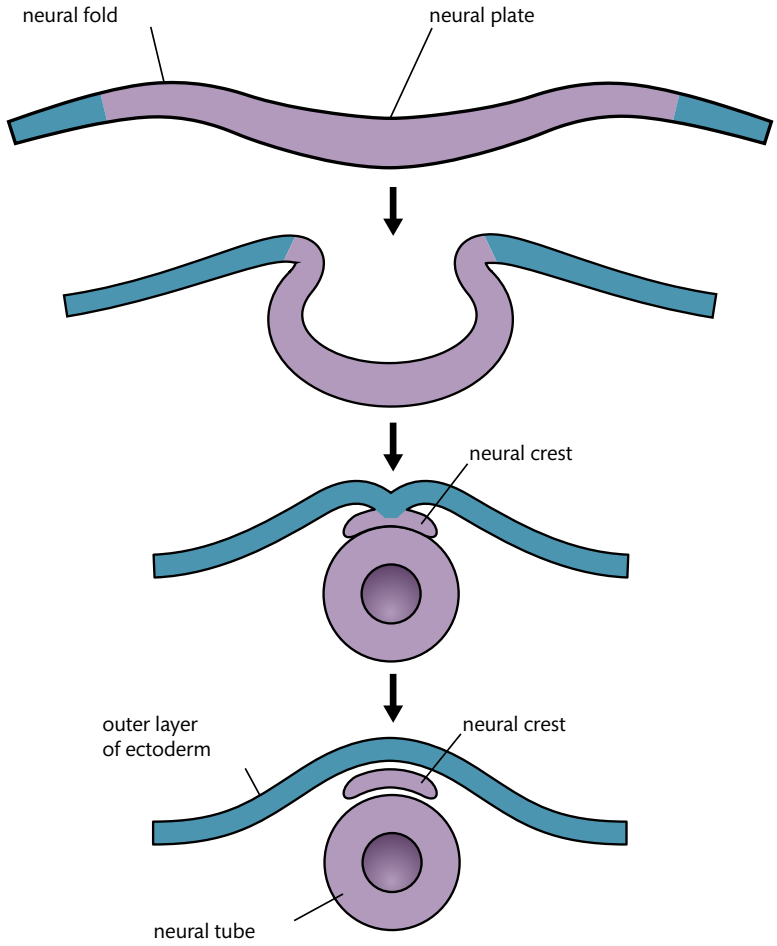


Figure 12.2 Formation of the neural tube from the neural plate. Campbell and Reece 1999

Spina bifida

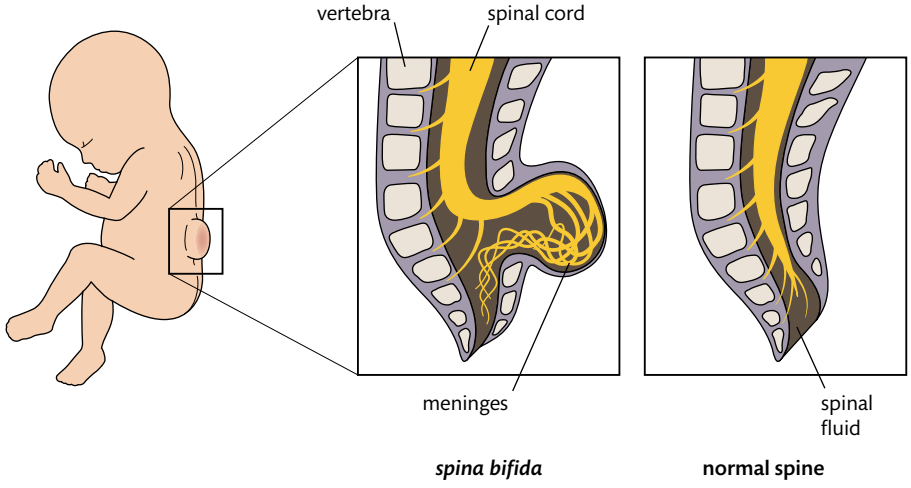
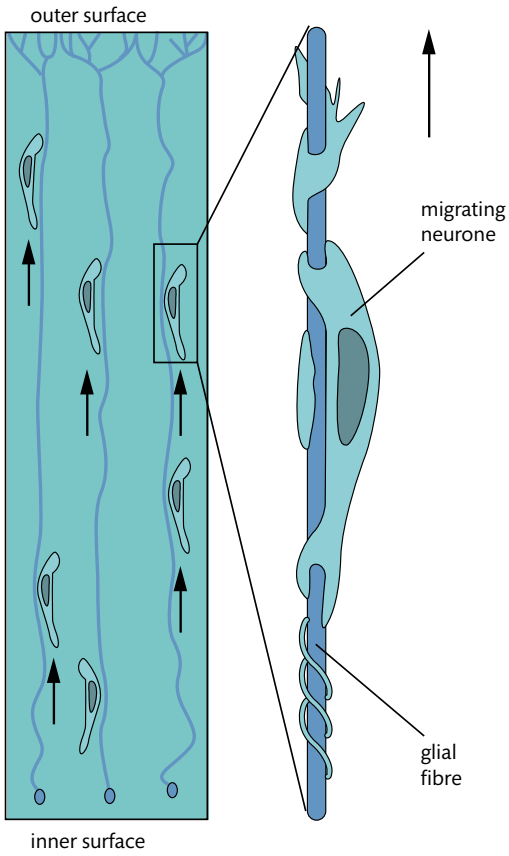


Figure 12.3 *Spina bifida* is caused by incomplete closing of the neural tube. http://babygilbertfund.com/

The closure of the neural tube does not take place simultaneously all along the body of the embryo. The area where the brain forms is well advanced over the caudal (tail) area. Closure of the neural tube in the tail area occurs more slowly and may not even completely close during embryonic development. This failure to close the human posterior (caudal) neural tube at day 27 of development results in the condition of *spina bifida*. How severe this is depends on how much of the spinal cord remains exposed.

Neurogenesis and migration of neurones

The neurones of the central nervous system (CNS) in the developing vertebrate embryo originate in the neural tube. Neuroblasts are immature neurones that are the precursor cells of neurones. The process of differentiation from neuroblast to neurone is called neurogenesis. As soon as the neural tube begins to transform into specific brain parts, two major families of cells begin to differentiate. These two types of cells are neurones and glial cells. Neurones carry messages, while glial cells do not carry messages. Ninety per cent of brain cells are glial and have many functions. One important function is physical and nutritional support of the neurone. Most of the new neurones in the human cortex are formed between the fifth week and the fifth month of development.



Glial cells provide a scaffolding network along which the immature neurones migrate. Along this scaffolding of the glial cells, immature nerve cells can migrate to their final location, mature, and send out their axons and dendrites.

Closure of the human neural tube seems to be controlled by a combination of genetic and environmental factors. Certain genes have been found to control the formation of the mammalian neural tube, but dietary factors also seem to be critical. The US health service recommends that women take supplemental folic acid during pregnancy to prevent neural tube defects. One estimate suggests that using vitamin B_{12} as a supplement can prevent 50% of neural tube defects.

Helen Cooper and her team at the Queensland Brain Institute have identified signalling molecules that may be used to promote the birth of new neurones, which will then migrate to damaged regions of the brain. This could be a major step forward in achieving functional recovery in a damaged brain.

Figure 12.4 Scaffolding glial cells allow neurones to reach their final destination.

'Glia' means glue in Greek. The word neurogenesis comes from 'neuro' meaning nerve cell and 'genesis' meaning beginning.

TOK

For more than 100 years scientists believed that glial cells did not play a role in neurotransmission. Only as recently as 2010 was this idea shown to be false. Why is the idea of falsification important to scientific knowledge?

Axon growth

As the neurone grows, it will send out one long axon moving towards a distant area. At the tip of the axon is a growth cone, which directs the axon. In cell cultures it is possible to watch axons grow. When an axon contacts an unfavourable surface it contracts, but with a favourable surface it persists. An axon can move forward at about 1 mm a day.

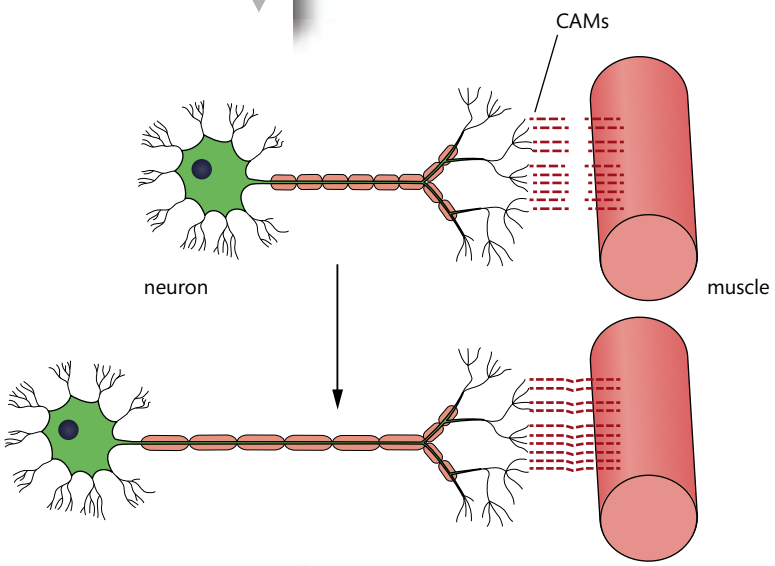
When neurones have reached their final location, synaptic connections must be made with their target cells. These target cells produce chemical messages that the neurone responds to. The signal molecule from the target cell can be secreted into the extracellular environment or carried on the target cell's surface. The neurone responds to the chemical messages by forming synapses with the target cell.

Neuroblasts differentiate into neurones. Neurones grow towards their target cells. The target cells give chemical signals, e.g. CAM, to the neurone.

Certain molecules from the target cell can act as signals to the growth cone. One type of signal molecule is called a cell adhesion molecule (CAM). CAMs are located on the surface of cells in the growth environment of the axon. The growth cone of the axon has a receptor called a CAM-specific receptor, so that when a CAM and its receptor recognize each other, chemical messaging takes place within the neurone. This results in the activation of enzymes within the neurone that contribute to the elongation of the axon.

Some receptors on a growth cone can also pick up the signal of molecules secreted by the target cell that diffuse into the extracellular environment. These are called chemotrophic factors. These factors can be attractive or repellent. Chemoattractive factors attract the axon to grow towards it. Chemorepellent factors repel the axon, so that the axon will elongate in a different direction. The growth cone responds to the various chemical stimuli that show it what path to follow and what connections to make.

Figure 12.5 Signal molecules called CAMs attract the axons to their target muscle cells.



Some axons extend beyond the neural tube

Some neurones, for example mammalian motor neurones, have to send their axons out of the area of the neural tube and travel much further in their journey towards other target cells. This gives the mammal the ability to control voluntary muscular movement. The motor neurones must extend their axons out of the CNS (the brain and spinal cord) in order to form these circuits. Newly developed motor neurones, which extend axons from the spinal cord, are some of the longest neurones in the body. During embryogenesis, these cells follow the same pathways to synapse with muscle targets as other neurones located within the CNS. The muscles that need to attract the axons will produce CAMs. The CAM receptor in the axon will activate enzymes to cause the growth cone of the axon to grow towards the muscle.

An axon that begins in the spinal cord and innervates a muscle in the foot can be as long as 1 m.

Multiple synapses

A huge number of synapses are formed during early brain development. Imagine if you could not remember your password and were desperate to download a movie. You would try all of the passwords you have ever used until one of them worked perfectly. A developing neurone does the same sort of thing, trying out all the possible connections to see which is the best fit. A single nerve cell can make a myriad of connections with its neighbouring nerve cells at the many points of branching that radiate from the main cell body. Not every cell will be the best partner. The job of the neurone is to find the best fit. In other words, only those synapses that have a function will survive, and the rest will gradually weaken until they disappear. Just think how easy it is to forget a password that you never use.

The neurones of the brain try to form a synapse with any nearby target cell, and then attempt to test out the connection. Will the connection work? Many do not, and those connections are eliminated. When the connections are between functionally compatible neurones, the result is a strengthening of communication.

Neuroscientist Z. Josh Huang, at Cold Springs Harbor Laboratory, in an article published in the *Journal of Neuroscience*, described the behaviour of neurones making tentative connections with almost every available partner. Lots of partners are tried, and eventually one is found that is compatible. Huang goes on to state that one mechanism at work during these rapid connections is controlled by a type of neural adhesion molecule that is recruited to the site of the connection. This adhesion molecule is also a type of CAM, called immunoglobulin CAM (IgCAM), and it acts like a lock and key. CAMs form a physical but reversible glue-like bond between the tentative projection of one cell's axon and the receiving structure on a neighbouring cell. Eventually, many of these connections are lost because it turns out they are not with the right partner cell.

Some synapses do not persist

Just as you would not use any passwords that do not work, the neurone will not keep any synapses that do not work. Most of our information about how the growth cones of an axon find their way to the target cell comes from the study of neurones that have travelled to a muscle from the spinal cord. Where they connect is called the neuromuscular junction. The axons form synapses that compete for the ability to innervate a muscle fibre. Specific molecules from the neurones and muscles facilitate these connections. The strongest connection will survive, and the rest are eliminated.

A muscle fibre is the site of a heated competition, with multiple synapses trying to win. Eventually, the connection made will be the best one between one motor neurone and the muscle fibre. As development proceeds, the other synapses are eliminated. Finally, the strength of the remaining synapse is increased. This is how the circuitry of the nervous system is formed.

Neural pruning

Pruning results in the overall number of neurones being reduced. When an infant is 2 or 3 years old, he or she has 15 000 synapses per neurone. This is twice as many as in an adult brain. Neural pruning eliminates axons that are not being used. The purpose of neural pruning seems to be to remove the simpler connections made in childhood and replace them with the more complex wiring made in adulthood. As

The word synapse is 113 years old. It was first coined in a textbook of physiology written in 1897. The author, Michael Foster, derived the word from the Greek words 'syn' and 'haptein', which mean together and clasp, respectively.

we have seen with other descriptions of neurone activity, pruning seems to follow the 'use it or lose it' principle. Synapses that are rarely used are eliminated, and those with strong connections are maintained. The removal of unneeded connections leads to improvement in brain efficiency.

Scientists supported by the National Institutes of Health in the USA have been studying pruning using the mouse as a model organism. They have discovered that cells called microglia, a type of glial cell, can prune unused synapses. This precise elimination of synapses that are unused and the strengthening of the more active synapses is a key part of normal brain development. Researchers hypothesize that microglia select a synapse for removal based on the inactivity of the synapse.

If a young child is deprived of stimulation, certain neurone pathways and synapses may be discarded. This is neural pruning. Synapses that are highly active will be preserved, while those that are underactive will be pruned. As we have seen, a 2–3-year-old child has the most synapses. Early childhood is the best time to learn language skills, when the excess synapses provide the raw material for the language experience to act on. Research into bilingualism suggests that exposure to more than one language is an excellent means of cognitive strengthening when young.

The plasticity of the nervous system

To learn more about brain plasticity, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.1.

Brain plasticity is the concept, now widely accepted, that the brain has the ability to change and adapt as a result of experience. Until 1960, researchers believed that only the brain of an infant or child could change, and that by adulthood the brain was unchangeable. Modern research has demonstrated that the adult brain does have plasticity. It can rewire itself after suffering massive strokes. Today we understand that the brain can create new neurones and new pathways. Scientists have shown that plasticity can vary with age, and that it is influenced by both environment and heredity. Thus we now know that the brain and nervous system are not static as previously thought.

The brain exhibits two types of plasticity: functional and structural. Functional plasticity is the ability of the brain to move functions from a damaged area to an undamaged area. Structural plasticity refers to the fact that the brain can actually change its physical structure as a result of learning.

An example of a functional shift can be illustrated by studying a tennis player who has suffered a stroke and has a paralysed left arm. During his rehabilitation, his good arm and hand are immobilized by the physical therapist, so that he can't use them. The tennis player is then given the task of cleaning tables. At first the task is impossible for him, but slowly his bad arm begins to remember how to move, and eventually he is back playing tennis. The functions in the brain areas that were killed by the stroke are transferred to healthy regions. New connections are formed between the intact neurones; these neurones are stimulated by activity.

An example of a structural shift in the brain is has been shown in a study of London taxi drivers by McGill University scientists. By observing London taxi drivers using magnetic resonance imaging (MRI) techniques to obtain images of their brains, the scientists discovered that experienced drivers have a larger hippocampus area in their brain than other drivers. This seems to be because their job needs their brain to store large amounts of information and to have good spatial understanding. London taxi drivers have to pass an extensive test on 320 standard routes throughout the city before they can start working. Most drivers prepare for the test over 34 months

by practising the routes on a moped. MRIs have shown a structural change in the hippocampus of these taxi drivers, which increases with the length of time a driver has been doing the routes.

Stroke may promote reorganization of brain function

Neuroimaging studies on stroke patients suggest that functional and structural reorganization of the brain takes place during recovery. This includes axon sprouting (new connections between axons), post-stroke neurogenesis (migration of new neurones to the site of the injury), differentiation of immature glial cells, and new associations with neurones and blood vessels.

Does the brain do this all by itself, or do we have some input into how this reorganization takes place? We know that after a stroke there are both chemical and physical changes in the pathways. What can be done to promote recovery?

In animal models with primates, it has been shown that improvement can be made with intervention. After a stroke resulting in weak hand movement in monkeys, the monkeys that did exercises with food rewards improved more rapidly than those that did not exercise. The part of the brain that improved shoulder movement took over the movement of the hand. The brain had reorganized itself in those monkeys that had received therapy.

In addition to animal models, new technologies have increased our knowledge of how the brain recovers from a stroke. Functional magnetic resonance imaging (fMRI), positron emission tomography (PET), brain mapping (magnetoencephalography, MEG) and other technologies have unravelled the many brain changes that take place in response to rehabilitation strategies and drugs. A common condition that results from a stroke is partial or complete loss of language function, called post-stroke aphasia (PSA). It had been estimated previously that the window for improvement of PSA was the first year following the stroke. Results of modern brain imaging studies have demonstrated that the recovery of language function can occur well beyond this period. These brain imaging and mapping techniques help clinicians and researchers design better strategies to enhance recovery.

Gregoire Courtine, who works at the Brain Mind Institute in Switzerland, has decided to switch the paradigm for those who have spinal cord damage and paraplegia. The switch is to change the view of the patient from a 'non-functioning person' to a 'person who is in a dormant state'. He describes his idea by imagining an injured patient as a car with all the parts (muscle, bone, etc.) present but the engine turned off. His goal is to produce a pharmaceutical cocktail to prepare the nerves for stimulation. Next he will surgically implant a mechanical object that will communicate between the brain and the spinal cord. Eventually the person will be able to move and walk again. He has called his research programme the 'rewalk' programme. How is this a new paradigm of how paraplegics are viewed?

To learn more about work with spinal cord injuries and see the work of Professor Courtine, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.1.

Two Mayo Clinic scientists have worked as a team to address the problem of regeneration of nerve tissue in spinal cord injury patients. Anthony J. Windebank, a neurologist, has implanted stem cells into damaged nerve tissue. He manipulated the stem cells so that they would promote nerve regeneration. The stem cells delivered the neural growth factors needed for nerve regeneration, but something else was needed for the spinal cord injury to be repaired. In order for the axons to find and connect with an appropriate target cell, a scaffold was needed. Michael J. Yaszemski, a biomedical engineer, was able to create such scaffolding. He designed tubing that acts as a synthetic, biodegradable scaffold. This scaffold can connect severed axons. Working together, these two scientists have pioneered a technique to insert stem cells into scaffold implants in injured spinal cords in animals. Eventually, this work will proceed to human trials.

Exercises

1 Describe spina bifida.
2 Outline the differentiation and migration of immature neurones.
3 Explain neural pruning.
4 Compare and contrast functional and structural plasticity of the brain.

Always include examples in answers to compare and contrast questions. Remember that compare and contrast means to give similarities and differences between two or more items, referring to both of them throughout.

NATURE OF SCIENCE

Use models as representations of the real world: the sensory homunculus and motor homunculus are models of the relative space human body parts occupy on the somatosensory cortex and the motor cortex.

A.2 The human brain

Understandings:

- The anterior part of the neural tube expands to form the brain.
- Different parts of the brain have specific roles.
- The autonomic nervous system controls involuntary processes in the body using centres located in the brainstem.
- The cerebral cortex forms a larger portion of the brain and is more highly developed in humans than other animals.
- The human cerebral cortex has become enlarged principally by an increase in total area with extensive folding to accommodate it within the cranium.
- The cerebral hemispheres are responsible for higher order functions.
- The left cerebral hemisphere receives sensory input from sensory receptors in the right side of the body and the right side of the visual field in both eyes, and vice versa for the right hemisphere.
- The left cerebral hemisphere controls muscle contraction in the right side of the body, and vice versa for the right hemisphere.
- Brain metabolism requires large energy inputs.

Applications and skills:

- Application: Visual cortex, Broca's area, nucleus accumbens as areas of the brain with specific functions.
- Application: Swallowing, breathing, and heart rate as examples of activities coordinated by the medulla.
- Application: Use of the pupil reflex to evaluate brain damage.
- Application: Use of animal experiments, autopsy, lesions, and fMRI to identify the role of different brain parts.
- Skill: Identification of parts of the brain in a photograph, diagram, or scan of the brain.
- Skill: Analysis of correlations between body size and brain size in different animals.

Guidance

- *Image of the brain should include the medulla oblongata, cerebellum, hypothalamus, pituitary gland, and cerebral hemispheres.*
- *Although specific functions can be attributed to certain areas, brain imagery shows that some activities are spread in many areas, and that the brain can even reorganize itself following a disturbance such as a stroke.*

The neural tube expands to form the brain

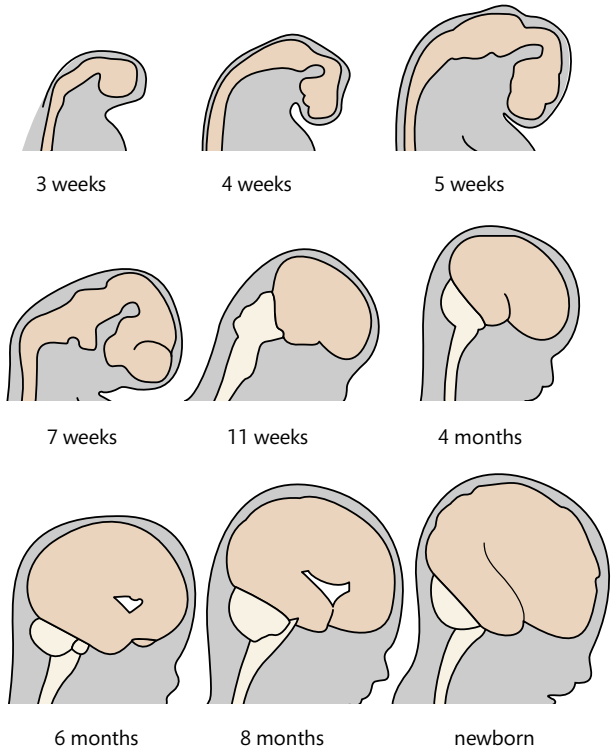


Figure 12.6 The neural tube expands to form the brain.

From the study of neurogenesis in Section A.1, you should now be familiar with the neural tube and how it is formed. To form the brain, nerve cells migrate to the outer edge of the neural tube and cause the walls to thicken. Eventually, the neural tube develops into the entire central nervous system: the brain and the spinal cord. The anterior end of the neural tube (forebrain) expands dramatically into the cerebral hemispheres. The posterior end of the neural tube develops into the other brain parts and the spinal cord. Neural development is one of the first systems to begin developing and one of the last systems to finish developing before birth. Brain development is one of the most complex systems in the embryo.

Researchers from the University of Texas Health Science Center in San Antonio have reported that eating less during early pregnancy impairs foetal brain development in a non-human primate model.

Different parts of the brain have specific roles

The brain is the most complex organ in the body. This jelly-like group of tissues, weighing 1.4 kg, produces our thoughts, feelings, actions, and memories. It contains an amazing 100 billion neurones, with thousands of synapses making the amount of connectivity literally mind-boggling. New connections are formed every day of our lives. These new connections store memories, learning, and personality traits. Some connections are lost and others are gained. No two brains are identical, and your brain continues to change throughout your life.

The brain regulates and monitors unconscious body processes such as blood pressure, heart rate, and breathing. It receives a flood of messages from the senses, and responds by controlling balance, muscle coordination, and most voluntary movement. Other parts of the brain deal with speech, emotions, and problem solving. Your brain allows you to think and dream.

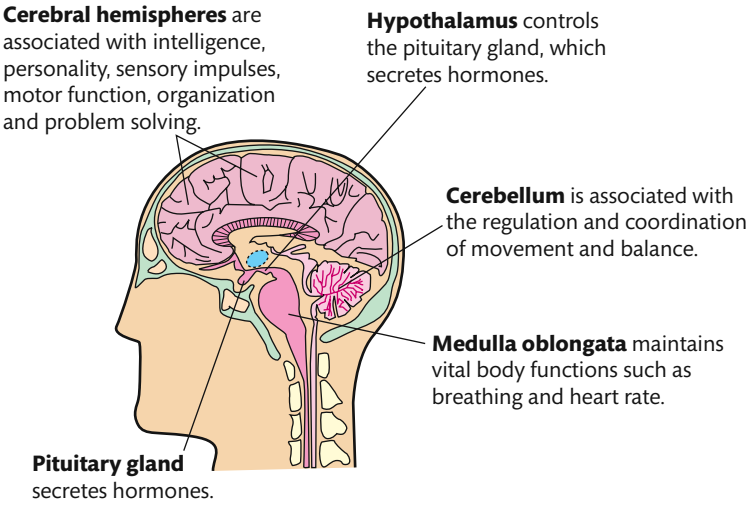


Figure 12.7 Parts of the human brain.

The following bullet points would be suitable text for annotating a diagram of the brain.

- Cerebral hemispheres act as the integrating centre for higher complex functions such as learning, memory, and emotions.
- The hypothalamus maintains homeostasis, coordinating the nervous and the endocrine systems. It synthesizes hormones which are stored in the posterior pituitary and releases factors regulating the anterior pituitary.
- The cerebellum is often called 'the little brain' because it has two hemispheres and a highly folded surface. It coordinates unconscious functions, such as movement and balance.
- The medulla oblongata controls automatic and homeostatic activities, such as swallowing, digestion, vomiting, breathing, and heart activity.
- The pituitary gland has two lobes, the posterior lobe and the anterior lobe. Both are controlled by the hypothalamus, and both secrete hormones regulating many body functions.

Role of the medulla

The medulla oblongata contains a 'swallowing centre' that coordinates the muscles of the mouth, pharynx (throat), and larynx (Adam's apple), so that a bolus of food will move down the oesophagus to your stomach during swallowing, and not down your windpipe (trachea).

The medulla oblongata controls breathing by monitoring the level of carbon dioxide in the blood. If there is an increase in carbon dioxide (meaning low oxygen), the rate and depth of breathing are increased so that more oxygen is take in.

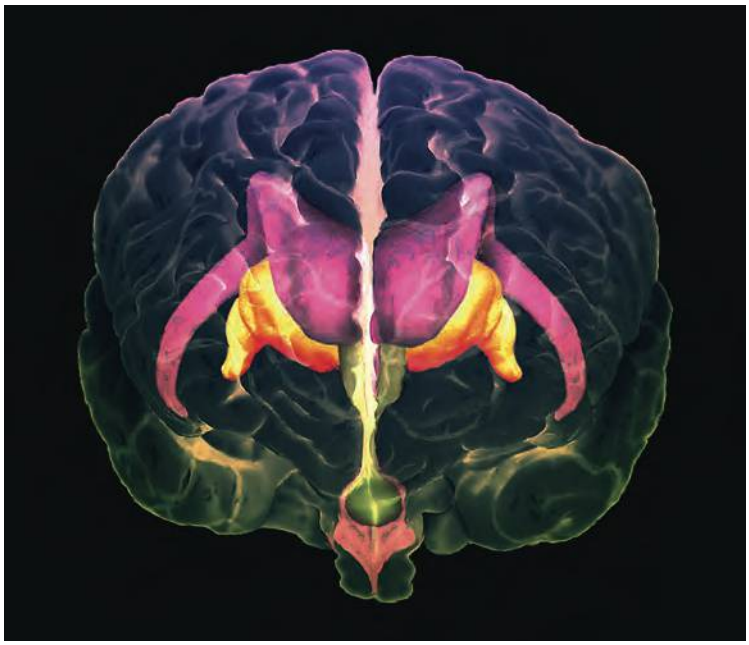
The medulla oblongata is also the cardiovascular centre for the body. The heart rate will slow down if it is activated by the cardioinhibitory centre or speed up if it is activated by the cardioaccelerator centre. When you first begin to exercise, the cardioinhibitory centre stops causing an increase in heart rate. During more strenuous exercise, the heart rate increases by direct stimulation of the cardioacclerator centre.

CHALLENGE YOURSELF

2 Study Figure 12.7, then try to draw a picture of the brain without looking at it.
- After you have drawn the picture, put on the labels.
- Every label must have a line leading exactly to the part it is referring to.
- Use straight lines and a ruler. Never use arrowheads when labelling. Only use arrows when you are writing about a process such as photosynthesis.
- Next annotate the diagram. Annotate means to write the function of each labelled part.

Identifying the role of different brain parts

The study of the complex information processing system that includes the brain and the nervous system is called neuroscience or neurobiology. New technology has provided us with valuable insights into the functions of our brains. Animal experimentation has allowed us to see exactly what causes some of our behaviour. Brain injuries have been studied to show what occurs when parts of the brain are damaged. Brain scans using fMRI have revealed the effects of addictive drugs on the brain. There are published studies of how pain is perceived, and how endorphins act as painkillers.



This is a coloured composite three-dimensional functional magnetic resonance imaging (fMRI) and computed tomography (CT) scan of the human brain, seen from the front. The ventricles (pink) circulate the cerebrospinal fluid, which cushions the brain. Beneath the ventricles lie the thalami (orange), and the hypothalamus (green, centre), which controls emotion and body temperature, and releases chemicals that regulate hormone release from the pituitary gland (the round green body at the lower edge).

Brain lesions

One method of studying the brain is to look at people who have had injuries to particular areas of their brain. Lesions in identifiable areas of the brain tell us indirectly about the function of those parts of the brain. Some lesions that have been studied were in either the right or the left half of the brain, and have provided us with information about the differences between the two halves.

The brain is divided into the left and the right hemispheres. These hemispheres are connected by a thick band of axons called the corpus callosum. The two hemispheres do not have exactly the same functions.

The left hemisphere contains areas important for all forms of communication. Left-hemisphere damage can result from a stroke (broken or blocked blood vessels in the brain). After left-hemisphere damage, patients may have difficulty speaking or doing complicated movements with their hands or arms. Deaf people who have had left-hemisphere damage may no longer be able to use sign language to communicate.

The right hemisphere is not involved in communication, although it does help us to understand words. It specializes in receiving and analysing the information that comes from all of our senses. When people have lesions in the right hemisphere, they have problems identifying faces and locating an object correctly in space. Such a patient

TOK

New technology to study the human brain has been advancing at a rapid rate. Does this new knowledge of the human brain have intrinsic value or is it a double-edged sword that can be used for good and bad? Just as nuclear physics might be used to develop cheap sources of energy or to make bombs, could human brain research have both good and bad consequences? What are some of the potential benefits of new technology that has been developed to study the human brain? What are some of the potential hazards?

might not be able to identify melodies, for example. The right hemisphere helps us understand what we hear and what we see.

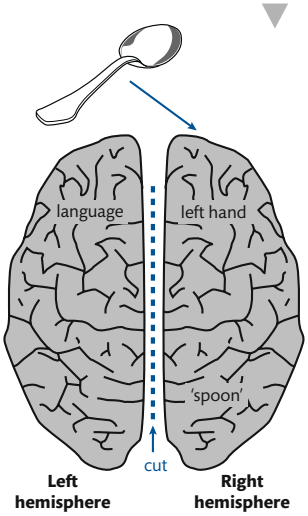
Early experiments with brain lesions were done in the mid-1800s with people who had particular injuries. Two neurologists observed that people who had injuries on the left side of the brain had speech and language problems. People who had injuries in the same areas but on the right side of the brain had no language problems. The two areas of the brain important for language are named after these scientists: Pierre Paul Broca and Carl Wernicke. Injury to the Broca's area interferes with the ability to vocalize words; injury to the Wernicke's area affects the ability to put words into sentences. Both areas are on the left side of the brain.

Another series of experiments was carried out in the 1960s. Scientists trying to find out about brain functions became interested in studying a group of patients who had undergone surgery to sever their corpus callosum to relieve symptoms of epilepsy. Experiments were devised to determine how splitting the brain affected these patients. Researchers already knew that input from the right visual field is received by the left hemisphere, and input from the left visual field is received by the right hemisphere.

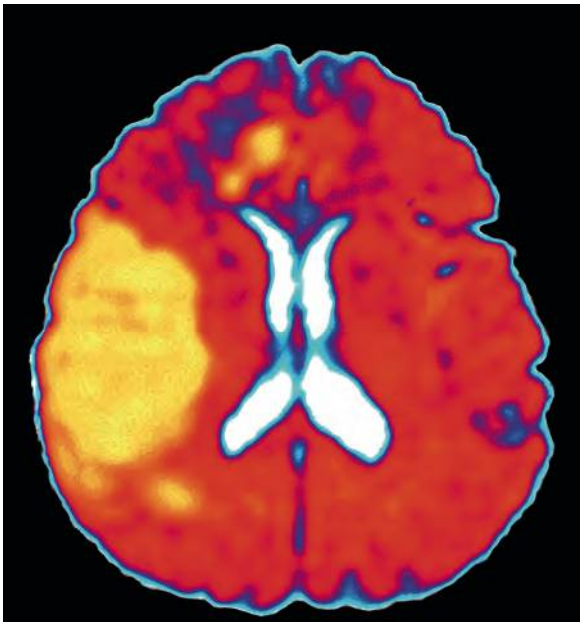
The scientists projected a picture of a spoon onto the right side of a card with a dot in the middle. If a split-brain person is sitting down looking at the dot and a picture of the spoon is flashed up, the visual information about the spoon crosses the optic chiasma and ends up on the left hemisphere. The person has no trouble identifying the spoon and says 'spoon'. (The language centre is in the left hemisphere.)

If the spoon is projected on the left side of the dot, the information goes to the right side of the brain, where there is no language ability (see Figure 12.8). In this case the person will say that he or she has seen nothing. Then the scientists asked the same person to pick up a spoon with his or her left hand. The subject correctly picks up the spoon. The verbal information travels to the right hemisphere, which understands what a 'spoon' is even if the word 'spoon' cannot be verbalized. If that person is then asked what is in his or her hand, he or she will not be able to say 'it is a spoon'. The right hemisphere has little language ability.

Figure 12.8 The split-brain experiment with a spoon.



fMRI scan of the brain of a patient after a stroke. The large area of yellow is a result of lack of blood flow to that area of the brain. The blockage of blood flow may be due to a blood clot. Strokes can cause the hemisphere in which they are located to lose function.



Functional magnetic resonance imaging (fMRI)

Functional magnetic resonance imaging (fMRI) uses radio waves and a strong magnetic field, not X-rays. This instrument enables scientists to see the blood flow in the brain as it is occurring. Researchers make movies of what is going on in the brain as a subject performs tasks or is exposed to various stimuli. This method can produce a new image every second. It can determine with some precision when regions of the brain become active and how long they remain active. This means it is possible to determine whether brain activity occurs in the same region or different regions at the same time as a patient responds to experimental conditions. A different tool called a positron emission tomography (PET) scanner is slower but has the advantage of being able to identify the areas of the brain activated by neurotransmitters and drugs. An fMRI is used by doctors to determine:

- a plan for surgery
- treatment for a stroke
- placement of radiation therapy for a brain tumour
- the effects of degenerative brain diseases such as Alzheimer's
- the diagnosis of how a diseased or injured brain is working.

Animal experiments

One type of relevant animal experimentation is to expose animal models to addictive substances in controlled situations. Animal models respond in similar ways to humans when addicted. Addicted animals:

- want more and more of the substance
- spend lots of time and energy getting the substance
- keep taking the substance despite adverse conditions
- have withdrawal symptoms upon withdrawal of the substance
- go back to the substance when stressed
- go back to the substance with another exposure to that substance.

To test whether a chemical meets the criteria for an addictive substance, a controlled self-administration experiment is designed and the response of the animal is recorded to see whether it fits the above model for addiction (see Figure 12.9).

1. An animal is trained to press a lever to get a reward.
2. The animal is given an injection of an addictive substance as it pushes the lever.
3. The lever will automatically give the injection if it is pushed by the animal (self-administration).
4. In order for this to be a controlled experiment, two levers must be available, one that gives the substance and one that does not (we want to be sure the animal is not just pushing the lever randomly).
5. If the substance is 'reinforcing', the animal will seek to repeat the experience by pushing that lever much more frequently. This would support the hypothesis that the substance is addictive.

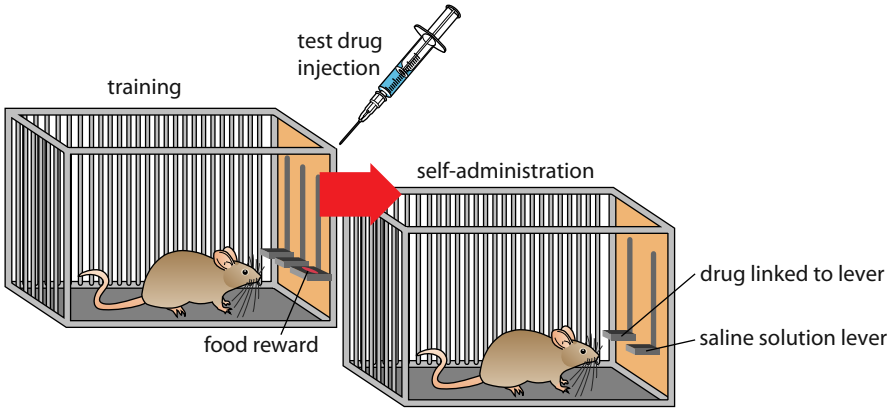


Figure 12.9 A self-administration experiment.

Researchers have recently used a self-administration experiment to support the hypothesis that acetaldehyde, which is a component of tobacco smoke, increases the addiction of adolescents to tobacco (see Figure 12.10).

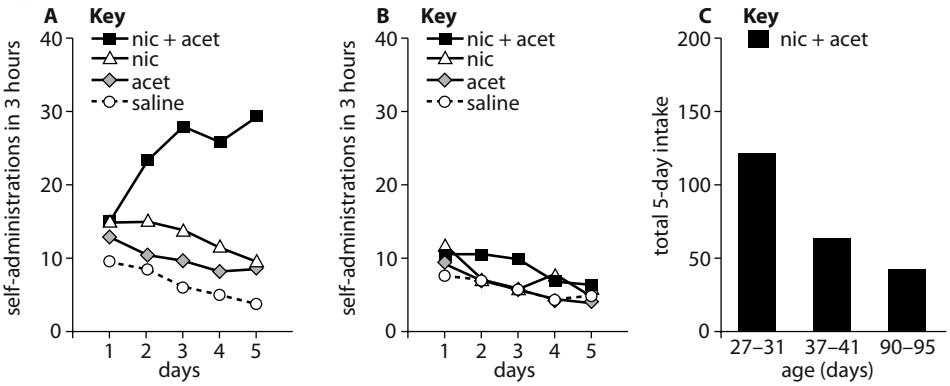


Figure 12.10 Adolescent rat experiments with nicotine and acetaldehyde. **(A)** Adolescent rats (27 days old) self-administered nicotine combined with acetaldehyde with increasing frequency over 5 days, but did not with nicotine alone, acetaldehyde alone or saline. **(B)** Adult rats (90 days old) did not demonstrate any preference. **(C)** The total 5-day intake of nicotine plus acetaldehyde was greatest for the youngest group of animals. This suggests that vulnerability to tobacco addiction decreases with age.

Animal experiments can shed light on the way that drugs promote abuse and addiction. Yet animal experiments can never replicate the complete picture of human interactions with drugs. Social factors are not considered in these experiments. Thus the results need to be interpreted with caution. Recent advances in technology have enabled researchers to use fMRI to answer questions that previously required an animal model.

A report released by the World Health Organization (WHO) claims that neurological disorders, such as Alzheimer's disease, epilepsy, stroke, headache, Parkinson's disease, and multiple sclerosis, affect 1 billion people worldwide.

TOK

According to Karl Popper, science is based on a series of theories. Scientific beliefs change over time, and it can be argued that a newer theory is closer to the truth than a previous theory. With enough evidence, there may be a paradigm shift as a new theory has more evidence to support it. Currently, the prevailing theory about the cause of Alzheimer's disease is that amyloid plaque accumulates on neurones. Alzheimer's disease results in extreme memory loss and affects millions of people worldwide. New research by Ben Barnes, published in the *Journal of Neuroscience* in 2013, is counter to the prevailing theory about Alzheimer's disease. The problem may not be the accumulation of plaque but an accumulation of a protein called C1q, which builds up on the synapse. Will there be a paradigm shift regarding the cause of Alzheimer's disease?

Autopsy

Autopsy can also be used to determine what brain parts are involved in certain functions. Paul Broca was a French surgeon who discovered the area of the brain involved in language. He autopsied the brain of a deceased patient who had a strange language disorder. The man was able to understand spoken language and could move his mouth and tongue, so he did not have motor impairment. However, he could not express his thoughts by writing or speaking. Following an autopsy of the man's brain, a lesion was discovered in the left inferior frontal cortex located in the left cerebral hemisphere. After studying the brains of eight other patients with similar disorders and finding the same lesions, Broca described this area of the brain in the left hemisphere as the language centre. This specific area in the left hemisphere is now called Broca's area and was the first area of the brain to be associated with a specific function.

The autonomic nervous system has two divisions

The brain is part of the central nervous system (CNS). The other part of the nervous system is the peripheral nervous system (PNS). The peripheral nervous system is considered to have two parts, the somatic system and the autonomic system (ANS). The somatic system takes sensory information from sensory receptors to the CNS and then sends back motor commands from the CNS to the muscles. The pain reflex arc is part of this system.

The ANS of the PNS is involuntary and regulates the activities of glands, smooth muscle, and the heart. Within the brain the ANS is located in the medulla oblongata. There are also two divisions to the ANS: the sympathetic system and the parasympathetic system.

CNS:

- brain
- spinal cord.

PNS:

- somatic (voluntary), information is received by the senses and messages sent to the skeletal muscles
- autonomic (involuntary), controls cardiac muscle of the heart, smooth muscle, and glands, consisting of two systems that are antagonistic
  - the sympathetic system
  - the parasympathetic system.

Table 12.2 The two PNS systems

Sympathetic system	Parasympathetic system
Important in an emergency	Important in returning to normal
Response is 'fight or flight'	Response is to relax
Neurotransmitter is noradrenaline	Neurotransmitter is acetylcholine
Excitatory	Inhibitory

As you can see, the sympathetic and the parasympathetic systems are antagonistic (see Figure 12.11). The sympathetic system is associated with 'fight or flight'. If you are facing an emergency, you need a quick supply of glucose and oxygen. The sympathetic system increases both the heart rate and the stroke volume (the amount of blood pumped by the left ventricle in each contraction) of the heart. It dilates the bronchi to give you more oxygen. It also dilates the pupil of the eye by making the radial muscles of the iris contract. Digestion is not necessary in an emergency, so the flow of blood to the gut is restricted by contraction of the smooth muscle of the blood vessels carrying blood to the digestive system (causing the diameter of the blood vessels to narrow).

If you are not in an emergency situation and are in a relaxed state, the parasympathetic system takes over. Parasympathetic nerves return the system to normal. The pupil of your eye constricts (gets smaller) to protect the retina, caused by contraction of the circular muscles of the iris. The heart rate slows and stroke volume is reduced. Blood flow returns to the digestive system. The smooth muscles of the blood vessels relax and the diameter of the blood vessels becomes wider.

To remember these confusing systems try to make sense of the terms. Peripheral is what is on the outside of the brain and the spinal cord. Somatic has to do with the body, and you know skeletal muscles are voluntary. Autonomic is similar to 'automatic', so you can remember that these functions are not voluntary. Sympathetic is when you are in 'sympathy' with your fear of a lion chasing you. Whereas with the parasympathetic system you are like a 'parrot' sitting up in a tree completely relaxed, because the lion is down on the ground.

If you can take the complex terms of biology and relate them to something else, you will find it easier to remember them.

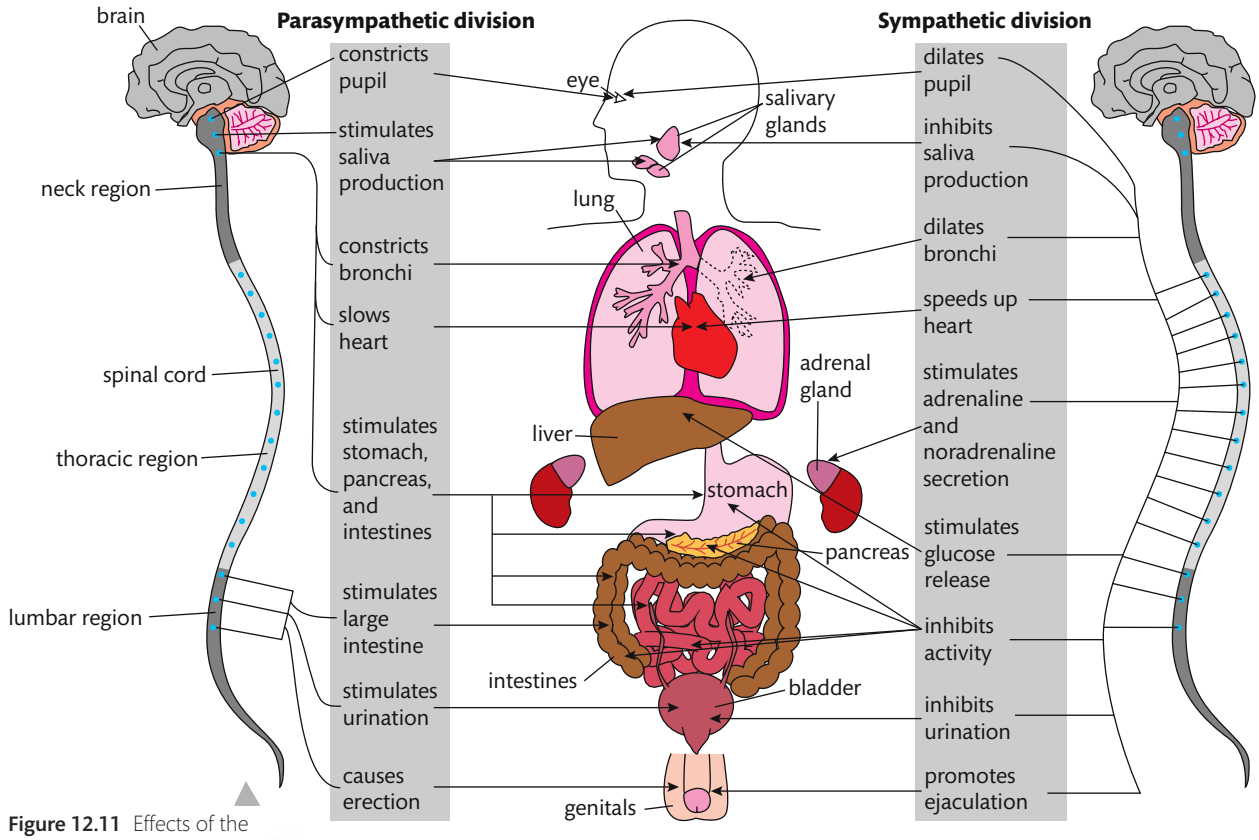


Figure 12.11 Effects of the autonomic nervous system.

The pupil reflex

In order to see the pupil reflex, ask someone to close their eyes and then suddenly open them (see Figure 12.12). You will see the pupil close in response to the sudden input of light as the eyes open. This is as much a reflex as the pain reflex. However, instead of having its connection in the spinal cord, as with the pain reflex, this is a cranial reflex. The sensory and motor neurones connect in the brain rather than the spinal cord.

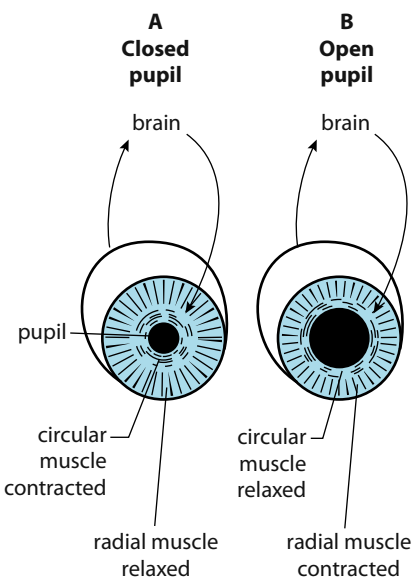


Figure 12.12 The pupil reflex.

In the eye, the iris surrounds the opening over the lens that we call the pupil. The iris contains two sets of smooth muscle to open and close the pupil like the aperture on a camera. The pupil closes as a result of a parasympathetic response caused by acetylcholine. If you go to an eye doctor, he or she may dilate your pupils by using a drug called atropine. Atropine stops the action of the neurotransmitter, acetylcholine. Constriction of the pupil happens because of a motor neurone causing the circular muscle to contract and so the radial muscle relaxes.

The pathway of the pupil reflex is shown in Figure 12.13 and described below.

- The optic nerve receives the messages from the retina at the back of the eye. The retina contains photoreceptors that receive the stimulus of light. Photoreceptors synapse with the bipolar neurones and then with the ganglion cells. Nerve fibres of the ganglion cells become the optic nerve.
- The optic nerve connects with the pretectal nucleus of the brainstem (the rectangle in Figure 12.13).

- From the pretectal nucleus, a message is sent to the Edinger–Westphal nucleus (the triangle in Figure 12.13), the axons of which run along the oculomotor nerves back to the eye.
- Oculomotor nerves synapse on the ciliary ganglion (the small circle in Figure 12.13)
- The axons of the ciliary ganglion stimulate the circular muscle of the iris, so it contracts.

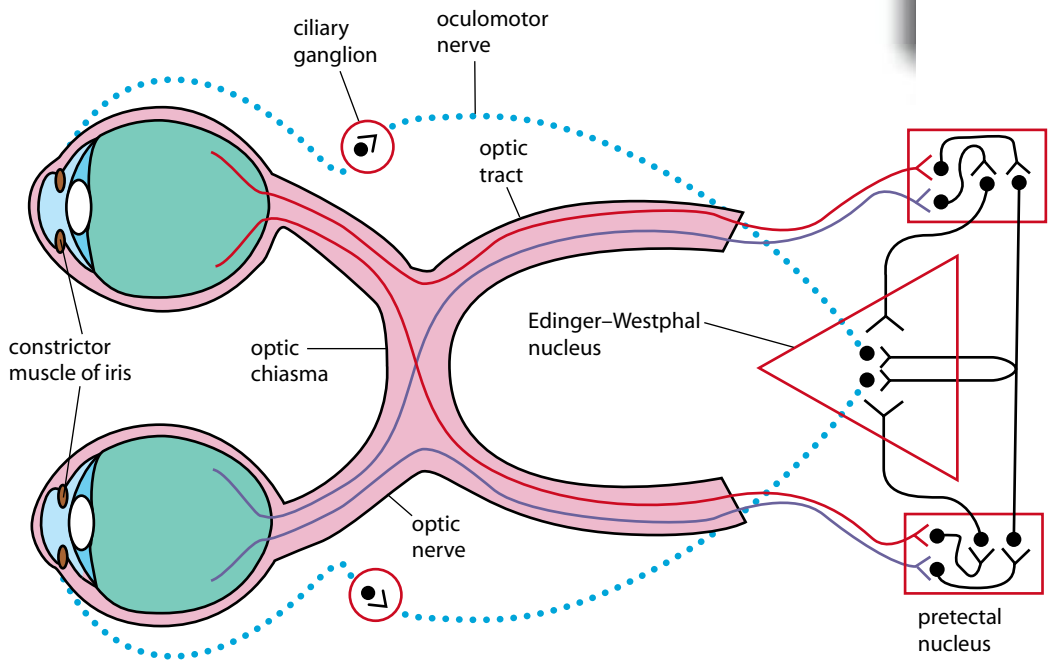


Figure 12.13 Parasympathetic pathways in the pupil reflex.

Brain death

As a result of recent advances in the treatment of patients, it is possible to artificially maintain the body without the impulses that normally come from the brain. The brainstem controls heart rate, breathing rate, and blood flow to the digestive system. The brain also controls body temperature, blood pressure, and fluid retention. All of these functions can be controlled for a patient without a functioning brain.

You may have heard news reports about patients who are living on life support systems but their brain shows no electrical activity. In some of these cases, family members may wish to keep the patient on life support because they do not believe that the person is dead. Other family members may believe that the person is dead, because the patient is 'brain dead'. What exactly does brain death mean?

The legal description of brain death is 'that time when a physician(s) has determined that the brain and brainstem have irreversibly lost all neurological function'. But people may still wonder if the patient could be in a coma. Patients in a coma have neurological signs that can be measured. These signs are based on responses to external stimuli. When examining for brain death, a physician must first perform a toxicology test to make sure that the patient is not under the influence of drugs that would slow down neurological reflexes. A diagnosis of brain death includes the following.

- Movement of extremities: if arms and legs are raised and let fall, there must be no other movement or hesitation in the fall.
- Eye movement: eyes must remain fixed, showing a lack of brain-to-motor-nerve reflex (as the head is turned there is no rolling motion of the eyes).
- Corneal reflex: this must be absent (when a cotton swab is dragged over the cornea, the eye does not blink).
- Pupil reflex: this must be absent (pupils do not constrict in response to a very bright light shone into both eyes).
- Gag reflex: this must be absent (the insertion of a small tube into the throat of a comatose patient will cause a gag reflex).
- Respiration (breathing) response: this must be absent (if the patient is removed from a ventilator, he or she does not breathe).

Following assessment by one or more physicians, a patient who shows none of these functions can be pronounced 'brain dead'. If the patient is missing all of the reflex responses and pupil responses, the evidence is clear that the brain will not recover.

However, in a brain-dead person there can still be spinal reflexes. The knee jerk response can still be functional. You may recall that the spinal reflexes do not involve the brain. In some brain-dead patients, a short reflex motion can still be exhibited if the hand or foot is touched in a certain manner.

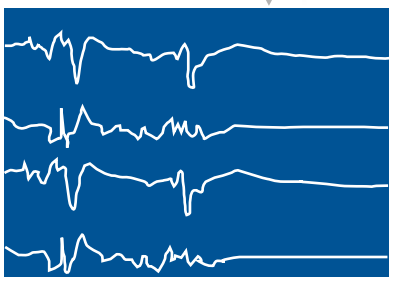


Figure 12.14 EEG showing activity followed by electrocerebral silence.

Many doctors order further tests in order to confirm brain death. Two tests commonly used are the electroencephalogram (EEG) and a cerebral blood flow (CBF) study. The EEG measures brain activity in microvolts. It is a very sensitive test. Some electrical activity will be shown on an EEG if a patient is in a deep coma. The lack of activity in a brain-dead patient is called electrocerebral silence (see Figure 12.14).

To measure blood flow to the brain, a radioactive isotope is injected into the bloodstream. A radioactive counter is then placed over the head for about 30 minutes. If no activity is detected, this is conclusive evidence of brain death.

To learn more about reflexes, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: A.2.

As you can see, the diagnosis of brain death is a very thorough process. At the end of the testing, there can be no doubt about the result. Once this diagnosis has been made, the patient may still be maintained on a ventilator, but a brain-dead person will not recover brain function.

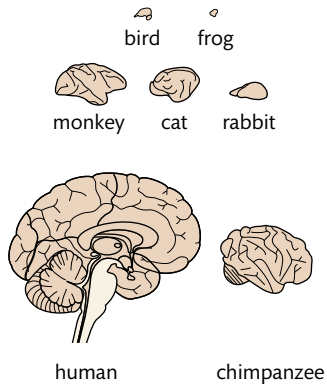


Figure 12.15 More intelligent animals have more highly developed cortical surfaces. https://faculty.washington.edu/chudler/brainsize.html

The cerebral cortex

The cerebrum develops from the front part of the neural tube. It is the largest part of the mature brain. As we have seen, the cerebrum consists of two divisions, the left and right cerebral hemispheres. The cerebral hemispheres are covered by a thin layer of grey matter (cells with no myelin sheath around them) called the cerebral cortex. This layer is less than 5 mm thick but contains 75% of the body's neurones. The cortex is where you perform tasks such as:

- reasoning
- language
- complex thought
- visual processing
- motor movement
- remembering
- speech.

The human brain is larger in proportion to its body size than brains of other animals. Orcas (killer whales) may have larger brains by actual volume, but when brain size is compared using a formula that takes body size into account, the human brain is three times as large as that of a chimpanzee and more than twice as large as that of an orca. The expansion of the human brain has come from the growth of the cerebral cortex.

The correlation between body size and brain size

The weight of the brain compared with the weight of the body is called the E:S ratio, where E stands for brain weight and S stands for body weight. Table 12.3 shows the E:S ratio for various species. You can see that humans and mice have the same E:S ratio, while the E:S for small birds is larger than that for humans. Should we therefore conclude that small birds, whose brains are comparatively larger in relation to their size compared with larger animals, are more intelligent than humans? You can see that the brain weight of a vertebrate does not appear to increase linearly with body weight. However, the trend seems to be that the larger an animal gets, the smaller its brain to body ratio. Small mice have a relatively large brain. Large elephants have a relatively small brain.

To improve on the simple ratio method, an equation was developed where E = weight of the brain, S = weight of the body, C = a constant, and r = an exponential constant. Using the formula $E = CS^r$, we can establish the relative capacity of brains of different species with different body weights.

When a value of C can be established for each species, then we can find the EQ or encephalization quotient: EQ = C/average mammalian value. For example, if the EQ of a certain species is 3.0 then the species has a value of C three times as high as a mammal of comparable weight with average encephalization (the ratio between actual brain size and predicted brain mass for an animal of a given size).

Look at the chart of EQ quotients (Table 12.4). A dolphin has an EQ of 5.31, which shows that it is twice as encephalized as a chimpanzee, which has an EQ of only 2.49.

Table 12.3 E:S correlation between the weight of the brain and the weight of the body for different animals
http://en.wikipedia.org/wiki/Brain-to-body_mass_ratio

Species	Simple brain-to-body ratio (E:S)
Small ants	1:7
Small birds	1:14
Human	1:40
Mouse	1:40
Cat	1:110
Dog	1:125
Squirrel	1:150
Frog	1:172
Lion	1:550
Elephant	1:560
Horse	1:600
Shark	1:2496
Hippopotamus	1:2789

Table 12.4 Encephalization quotient (EQ) data
http://serendip.brynmawr.edu/bb/kinser/Int3.html

Species	EQ	Species	EQ
Man	7.44	Cat	1.00
Dolphin	5.31	Horse	0.86
Chimpanzee	2.49	Sheep	0.81
Rhesus monkey	2.09	Mouse	0.50
Elephant	1.87	Rat	0.40
Whale	1.76	Rabbit	0.40
Dog	1.17		

CHALLENGE YOURSELF

We can agree that if the brain weight is large relative to the body (EQ), the animals may be able to accomplish more complex tasks. However, other factors may also be involved. See if you can answer the following questions using the EQ values shown in Table 12.4.

3 Can you see which four animals have diets of meat or fish?

4 Suggest why this diet is beneficial to the species.

5 Which three animals have a plant and insect diet?

6 Why is this diet not as beneficial to a species?

7 Can you think of any animal behaviour that would place a species high on the encephalization scale?

8 Why are rat and rabbit at the bottom in comparison with the other species?

9 Can you guess the name of an invertebrate animal that has a high EQ?

The enlargement of the cerebral cortex

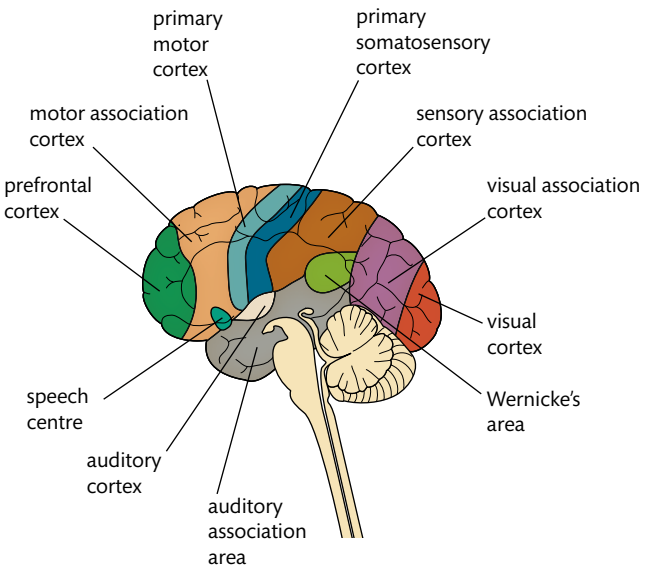


Figure 12.16 Functional divisions of the cerebral cortex. https://faculty.washington.edu/chudler/functional.html

Greater cognitive ability and more advanced behaviour are associated with an increase in size of the cerebral cortex. When we compare human brains with those of other animals, the biggest difference appears to be in the surface area of the cerebral hemispheres. In a mouse, for example, the surface of the cerebral cortex is smooth, while in a dog it is very convoluted. When we study monkeys and apes, even more folds are found in their cortex. In order for the brain to fit into a skull that is actually in proportion to the body, the brain has to fold in on itself. An increased surface area is needed for more complex behaviours, but it still has to fit into the limited space of a skull. One way to have more working surface is to add folds to the surface. If you scrunch up a sheet of A4 paper, it has the same surface area but can take up less space than a flat piece of A4 paper. As species evolved to be able to do more complex behaviours, they had to develop more working area for their brain. The more folding, the more surface area there can be. In this way a larger surface area of cerebral cortex can be contained in a limited space.

A 6-month-old foetus has a completely smooth cerebral cortex. By birth its brain has become the walnut-like structure we would expect to see. The folding of the cerebral cortex during development of the human embryo takes place during the last 3 months of development.

Functions of the cerebral cortex

The extensive folding of the cerebral cortex and the large numbers of neurones present in the cortex are evidence of the importance of this brain part. The higher order functions performed by the cerebral cortex are shown in Table 12.5.

Table 12.5 Functional areas of the cerebral cortex

Part	Function
Prefrontal cortex	Organizes thoughts, solves problems, and formats strategies
Motor association cortex	Coordinates movement
Primary motor cortex	Plans and executes movements
Primary somatosensory cortex	Processes information related to touch
Sensory association cortex	Processes sensory information of perceptions or multisensory information
Visual association area	Processes visual information
Visual cortex	Recognizes visual stimuli
Wernicke's area	Understands written and spoken language
Auditory association area	Processes auditory information
Auditory cortex	Detects sound quality such as loudness or tone
Broca's area	Produces speech and language

The visual cortex, Broca's area, and nucleus accumbens

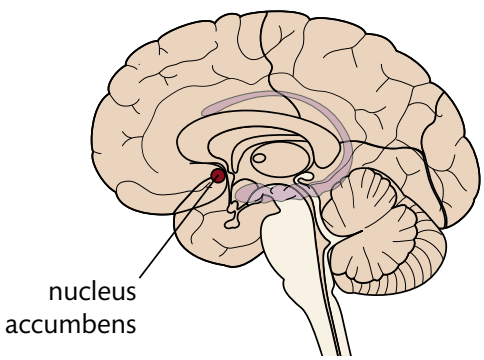


Figure 12.17 Nucleus accumbens is associated with the rewards circuit in the brain.

Broca's area is one of two parts of the cerebral cortex linked to speech and language (the other is Wernicke's area). Broca's area is labelled 'speech centre' in Figure 12.16. When a patient has a brain injury causing lack of language production, it is called Broca's aphasia. Paul Broca discovered the language function of this area.

The nucleus accumbens (see Figure 12.17) is associated with the reward circuit in the brain. It responds chiefly to two neurotransmitters: dopamine and serotonin. Dopamine promotes desire, while serotonin inhibits desire. The activation of dopamine in the nucleus accumbens is associated with the anticipation of a reward.

Drugs such as cocaine and nicotine also increase dopamine production in the nucleus accumbens. The reward of increased dopamine can result in addiction.

The visual cortex is the part of the brain that receives information from the cells in the retina of the eye. The visual cortex (see Figure 12.16) is one of many brain centres that cooperate to produce vision.

The left cerebral hemisphere receives sensory information from the right side of the body, and vice versa

The cerebral cortex is the thin layer on the surface of the left and right cerebral hemispheres and is responsible for all higher order functions. The cerebral cortex is made up of unmyelinated neurones and is called grey matter . The two cerebral hemispheres are connected by a thick band of tissue called the corpus callosum, through which communication takes place between the right and left sides of the brain. The corpus callosum is made up of myelinated neurones and is called white matter. Each cerebral hemisphere is responsible for one half of the body. The left cerebral hemisphere receives sensory input from sensory receptors on the right side of the body and the right side of the visual field in both eyes, and vice versa for the right hemisphere.

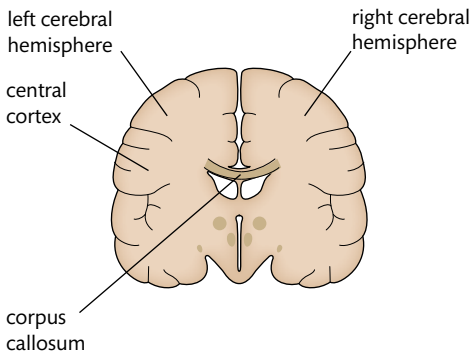


Figure 12.18 The corpus callosum connects the two cerebral hemispheres.

Each side of the cortex is divided into further sections depending on the activity that it performs. For example, look at Figure 12.16 and you can see the primary somatosensory cortex and the motor cortex. The primary somatosensory cortex is the main area for receiving the sense of touch. Sensory input from the right hand is sent to the left primary somatosensory cortex, and vice versa.

The neural pathways for vision travel to the primary visual cortex. As you might expect, the right side of the brain receives information from the left visual field, and the left side of the brain receives information from the right visual field. Looking at Figure 12.20, you can see that the optic nerves from the left field, shown in

NATURE OF SCIENCE

Look at the diagram of a cross-sectional map of the primary somatosensory cortex (Figure 12.19).

The relative space that the human body parts occupy in the sensory cortex can be illustrated by a 'cartoon-like' homunculus (man). In this picture you can see the homunculus, a distorted model that reflects the relative space that human body parts occupy in each cortex. Notice that the head occupies a large area but the hips and legs only occupy a small area. Sensory information from the head is much more important and has more brain space available to it than sensory information from the hip or leg. What seems to be more important in the motor cortex, the head or the knee?

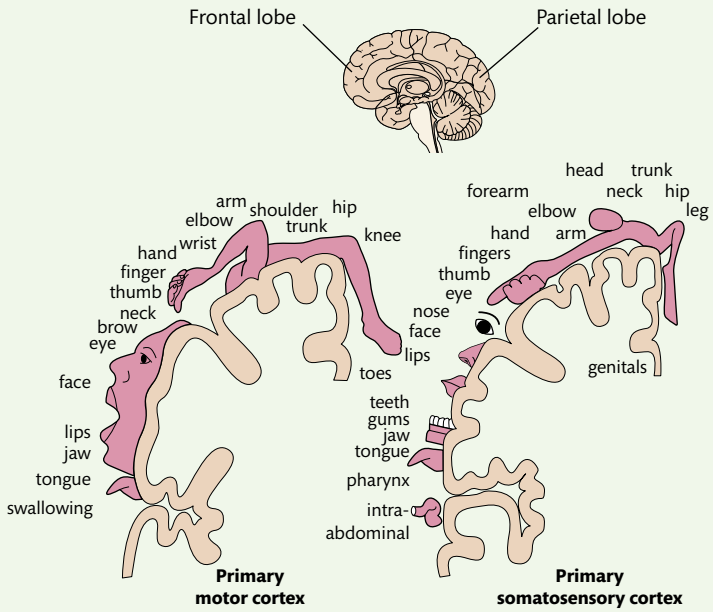


Figure 12.19 This homunculus cartoon shows the relative importance of areas of the body in the primary motor cortex and the primary somatosensory cortex.

Practise drawing diagrams of the neural pathway for vision until you understand it thoroughly.

blue, can cross over at the optic chiasm so that the information from left field of view is received by right side of the brain, and vice versa.

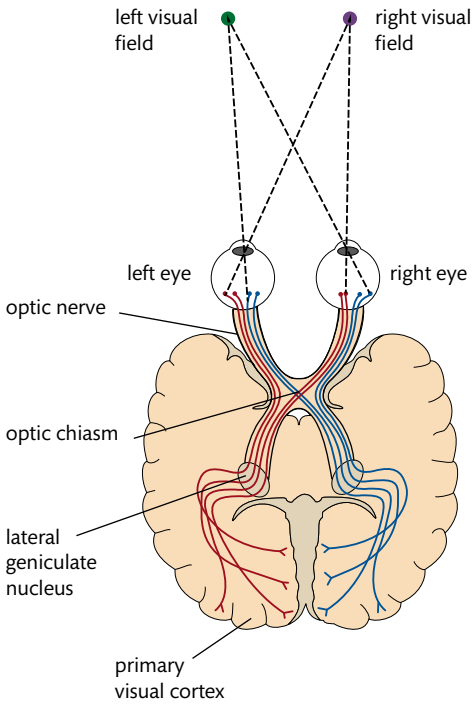


Figure 12.20 Two eyes connected to the brain by optic nerves. The left visual field is giving information to the right side of the primary visual cortex (note the blue lines). The right visual field is giving information to the left side of the primary visual cortex (note the red lines).

The left cerebral hemisphere controls muscle contraction in the right side of the body, and vice versa

Now let's look at the motor cortex. The motor cortex controls voluntary movements. As you can guess, the motor cortex in the right cerebral hemisphere controls movement

The hand area of the primary motor cortex is known to be larger among professional pianists than among amateur pianists. Peter L. Strick, from the Department of Neurobiology, Pitt School of Medicine, suggests that this indicates that extensive practice induces changes in the primary motor cortex.

on the left side of the body, and vice versa. This can be very obvious in patients who have had a stroke.

When a person has a stroke, it is localized and often occurs in either the left or right cerebral hemisphere. A stroke is caused by a blocked or ruptured blood vessel. This interrupts oxygen flow to the brain cells. If a motor area of the cerebral hemisphere on the left side of the brain is affected, then paralysis will be seen in the right arm and right leg. The location of the paralysis tells the doctor which side of the brain has been injured as a result of loss of oxygen. Fortunately, because the brain has plasticity, other parts of the brain may take over during rehabilitation and facilitate the return of full motion.

Brain metabolism requires large energy inputs

Neurones have a high energy need because they are always in a state of high metabolic activity. As you will recall, metabolism consists of all the chemical activities performed by a cell. Neurones perform many tasks that are similar to other cells, such as repairing or rebuilding their structural components. However, the chemical signals that are responsible for the communication between neurones consume half of all the energy used by the brain. This is why a brain cell needs twice the amount of energy as any other cell in a body.

Glucose is the primary energy source that fuels the metabolism of neurones in the human brain. Neurones cannot store glucose, so the blood must deliver a constant supply. Because of its high rate of metabolism, glucose is used up rapidly by neurones during mental activity. In an experiment on rats, scientists at the University of Illinois College of Medicine found that young rats have a good supply of glucose to the area of the brain involved in learning and memory, while older rats to do not have such a good supply. The supply of glucose for older rats runs out much more quickly. When glucose runs out, the behaviour of the older rats indicates that there is a large deficit in learning.

Blood sugar (glucose) is supplied by the food that you eat. High-quality carbohydrates, such as fruits, vegetables, legumes, grains, and dairy, are the best source of glucose. These foods provide the brain with a supply of glucose that lasts for hours. Food such as sugar snacks and drinks provide glucose quickly, but the supply does not last as long and can result in low brain activity. It has been seen in animal models that sustained levels of glucose in the brain are beneficial for learning.

Angelman's syndrome is diagnosed from well-recognized abnormal patterns on an electroencephalogram (EEG). Symptoms include developmental delay of 6–12 months in babies, in addition to facial abnormalities and seizures. Angelman's syndrome is a genetically inherited disorder, which primarily affects the nervous system.

Exercises

5 Draw and annotate a diagram of the human brain.

6 Describe how an fMRI is used to identify the role of different brain parts.

7 State the specific function of each of the following: Broca's area; nucleus accumbens; visual cortex.

8 Explain how folding has allowed the cerebral cortex to become more highly developed in humans than in other animals.

9 Explain why brain metabolism requires a large input of energy.

A.3 Perception of stimuli

Understandings:

- Receptors detect changes in the environment.
- Rods and cones are photoreceptors located in the retina.
- Rods and cones differ in their sensitivities to light intensities and wavelengths.
- Bipolar cells send the impulses from the rods and cones to ganglion cells.
- Ganglion cells send messages to the brain via the optic nerve.
- The information from the right field of vision from both eyes is sent to the left part of the visual cortex and vice versa.
- Structures in the middle ear transmit and amplify sound.
- Sensory hairs of the cochlea detect sounds of specific wavelengths.
- Impulses caused by sound perception are transmitted to the brain via the auditory nerve.
- Hairs in the semicircular canals detect movement of the head.

Applications and skills:

- Application: Red–green colour blindness as a variant of normal trichromatic vision.
- Application: Detection of chemicals in the air by the many different olfactory receptors.
- Application: Use of cochlear implants by deaf patients.
- Skill: Labelling a diagram of the structure of the human eye.
- Skill: Annotation of a diagram of the retina to show the cell types and the direction in which light moves.
- Skill: Labelling a diagram of the structure of the human ear.

Guidance

- *Humans' sensory receptors should include mechanoreceptors, chemoreceptors, thermoreceptors, and photoreceptors.*
- *Diagram of human eye should include the sclera, cornea, conjunctiva, eyelid, choroid, aqueous humour, pupil, lens, iris, vitreous humour, retina, fovea, optic nerve, and blind spot.*
- *Diagram of retina should include rod and cone cells, biopolar neurones, and ganglion cells.*
- *Diagram of ear should include pinna, eardrum, bones of the middle ear, oval window, round window, semicircular canals, auditory nerve, and cochlea.*

NATURE OF SCIENCE

Understanding of the underlying science is the basis for technological developments: the discovery that electrical stimulation in the auditory system can create a perception of sound resulted in the development of electrical hearing aids and ultimately cochlear implants.

Sensory receptors and diversity of stimuli

Certain foods can make you feel comforted. Seeing a familiar face in a crowd can make you feel at ease. Listening to your favourite music can make you feel happy. We have learned to link certain tastes, sights, and sounds with emotions. Sensory cells send messages to certain parts of the brain that control emotion and memory.

Taste and sound are not just for pleasure. They also protect us. We remember the taste of mouldy food. We move out of the way when we hear a car coming. Many lives have been saved by smelling smoke.

Sense organs are the windows to the brain. They keep the brain aware of what is going on in the outside world. When stimulated, the sense organs send a message to the central nervous system. The nerve impulses arriving at the brain result in sensation. We actually see, smell, taste, and feel with our brain rather than our sense organs.

We link certain tastes to emotion and memory. Some foods make us remember our childhood.

Receptors detect changes in the environment

Mechanoreceptors

Mechanoreceptors are stimulated by a mechanical force or some type of pressure. The sense of touch is caused by pressure receptors that are sensitive to strong or light

pressure. In our arteries, pressure receptors can detect a change in blood pressure. In our lungs, stretch receptors respond to the degree of lung inflation. We can tell the position of our arms and legs by the use of proprioceptors found in muscle fibres, tendons, joints, and ligaments. These receptors help us maintain posture and balance. In our inner ear, there are pressure receptors sensitive to the waves of fluid moving over them. This gives us information about our equilibrium.

Chemoreceptors

Chemoreceptors respond to chemical substances. Using this type of receptor, we can taste and smell. They also give us information about our internal body environment. Chemoreceptors in some blood vessels monitor pH changes. Changes in pH signal the body to adjust the breathing rate. Pain receptors are a type of chemoreceptor that respond to chemicals released by damaged tissues. Pain protects us from danger. The pain reflex makes us pull away, for example, from a hot object. Olfactory receptors respond to smell.

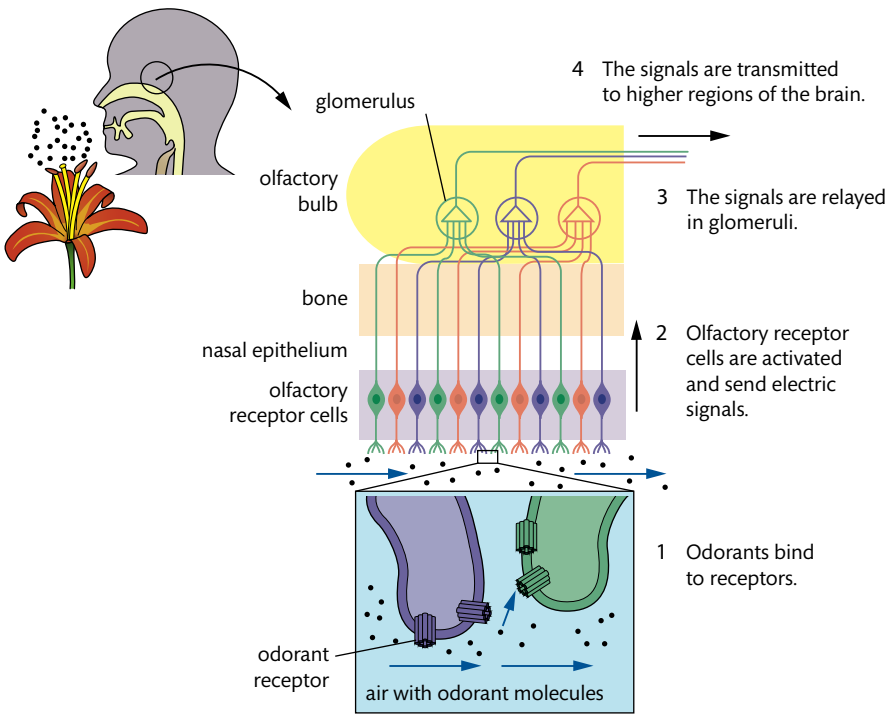


Figure 12.21 How receptors function in the olfactory system.

How does smell work? Everything you can smell, like bread baking, onions, coffee, anything good or bad, is releasing volatile molecules that diffuse into the air. These molecules then reach the olfactory receptors in your nose: 10 000 different smells can be detected by these receptors in humans.

At the top of your nasal passage is a patch of specialized neurones that contain the olfactory receptors. A given molecule may stimulate more than one receptor. The combination of several receptors is registered by the brain as a certain smell. Scientists have hypothesized that each of our hundreds of olfactory receptors is encoded for by a specific gene. Each specific gene recognizes a different smell. If your DNA does not have a certain gene, you may be unable to smell a certain smell. For example, some people cannot smell digested asparagus but others can.

Thermoreceptors

Thermoreceptors respond to a change in temperature. Warmth receptors respond when the temperature rises; cold receptors respond when the temperature drops. Human thermoreceptors are located in the skin.

Photoreceptors

Photoreceptors respond to light energy; they are found in our eyes. Our eyes are sensitive to light and give us vision. Rod cells in our eyes respond to dim light, resulting in black and white vision; cone cells respond to bright light, giving us colour vision.

The structure and function of the human eye

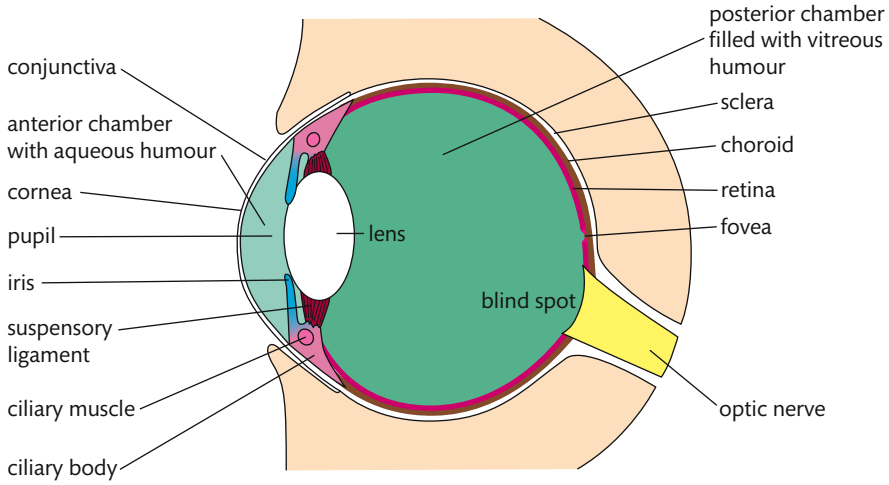


Figure 12.22 The human eye.

CHALLENGE YOURSELF

10 Many pictures are available online that you can use to practise labelling the parts of the eye. Print out a picture with missing labels and practise labelling the eye until you are sure you have learned all the parts perfectly. Use the hotlinks at the end of this section to find a diagram you can label as an interactive task. Next learn the function by covering up one side of Table 12.6 with a piece of paper and trying to recreate it.

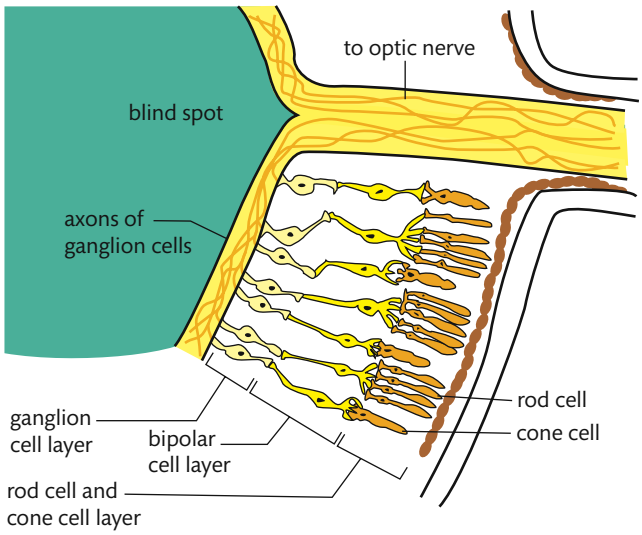


Figure 12.23 Structure of the retina.

TOK

We depend on more than our senses to know the biological world. To what extent are we dependent on technology for our knowledge of biology? As an example, we use molecular tools to read the basic instructions of life one letter at a time as we decode the human genome. What other ways can you think of in which we use technology to know the biological world?

Vision 2020 is a joint initiative of the World Health Organization and the International Agency for the Prevention of Blindness, whose goal is to eliminate avoidable blindness worldwide by the year 2020. Vision 2020 wants to give everyone in the world the right to sight.

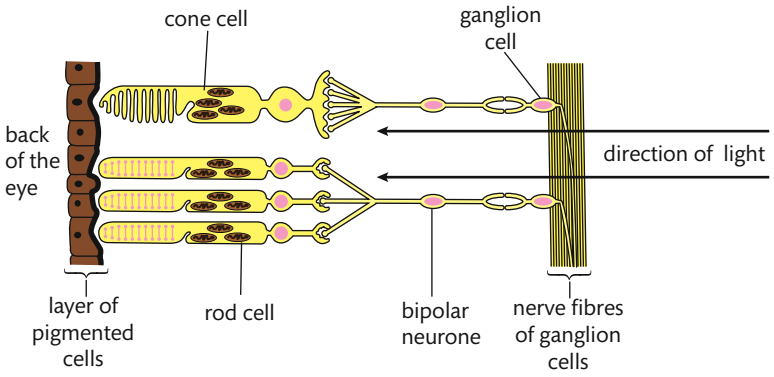


Figure 12.24 Structure and function of the retina.

Table 12.6 summarizes the functions of the various parts of the eye.

Table 12.6 The functions of various parts of the eye

Part	Function
Iris	Regulates the size of the pupil
Pupil	Admits light
Retina	Contains receptors for vision
Aqueous humour	Transmits light rays and supports the eyeball
Vitreous humour	Transmits light rays and supports the eyeball
Rods	Allow black and white vision in dim light
Cones	Allow colour vision in bright light
Fovea	An area of densely packed cone cells where vision is most acute
Lens	Focuses the light rays
Sclera	Protects and supports the eyeball
Cornea	Focusing begins here
Choroid	Absorbs stray light
Conjunctiva	Covers the sclera and cornea and keeps the eye moist
Optic nerve	Transmits impulses to the brain
Eye lid	Protects the eye

The retina

Vision begins when light enters the eye and is focused on the photoreceptor cells of the retina (see Figure 12.23). The photoreceptor cells are the rods and the cones. Notice in Figure 12.24 that both the rods and cones synapse with their own bipolar neurones. Each bipolar neurone synapses with a ganglion cell. The axons of the ganglion cells make up the optic nerve, which carries the message of vision to the brain.

The following bullet points would be suitable for annotating a diagram of the retina.

- Rod cells are photoreceptor cells that are very sensitive to light. They receive the stimulus of light, even very dim light, and synapse with a bipolar neurone.

- Cone cells are photoreceptor cells that are activated by bright light. They receive the stimulus of bright light and synapse with a bipolar neurone.
- Bipolar neurones are cells in the retina that carry impulses from a rod or a cone cell to a ganglion cell of the optic nerve. They are called bipolar because they each have two processes extending from the cell body.
- Ganglion cells synapse with the bipolar neurones and send the impulses to the brain via the optic nerve.

The steps of the vision pathway in the retina are as follows.

- **Rods and cones receive the light stimulus.**
- **Rods and cones synapse with the bipolar neurone.**
- **The bipolar neurone carries the impulse to the ganglion cell.**
- **The ganglion cell is located in the optic nerve.**
- **The optic nerve carries the impulse to the brain.**

Rods and cones

Table 12.7 provides a comparison of rods and cones.

Table 12.7 Rods and cones

Rods	Cones
These cells are more sensitive to light and function well in dim light	These cells are less sensitive to light and function well in bright light
Only one type of rod is found in the retina. It can absorb all wavelengths of visible light	Three types of cone are found in the retina. One type is sensitive to red light, one type to blue light, and one type to green light
The impulses from a group of rod cells pass to a single nerve fibre in the optic nerve (see Figure 12.23)	The impulse from a single cone cell passes to a single nerve fibre in the optic nerve (see Figure 12.24)

Red–green colour blindness

Normal vision uses the three classes of cones, red, green, and blue, and is called trichromatic vision. Some individuals are dichromatic and have red–green colour vision defects; dichromatic vision is a variant of trichromatic vision. Red–green defects are inherited as a sex-linked trait: sons can inherit the defect from their mother. It is very rare for females to have this trait. Dichromatic vision can be caused by the presence of blue and green cones with no functional red cones (red-blindness), or by the presence of blue and red cones with no green cones (green-blindness). Dichromats see the world differently depending on the variation they have inherited. Many websites have tests for colour blindness.

Information from the right field of vision from both eyes is sent to the left part of the visual cortex, and vice versa

Review the information in Section A.2 on the human brain and the visual cortex.

CHALLENGE YOURSELF

11 The next challenge is to learn the parts of the retina using Figure 12.24. The best way to learn this picture is to draw it and label the parts. Be accurate. Notice that the cone cell has only one part, while the rod cell has three. Make sure you understand where the back of the eye is, and the direction from which the light is coming. You can use Figures 12.23 and 12.24 so that you can really understand what is happening. To test yourself another way, try to explain the retina to someone else using the picture.

To complete a test for colour blindness, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.3.

The structure of the ear

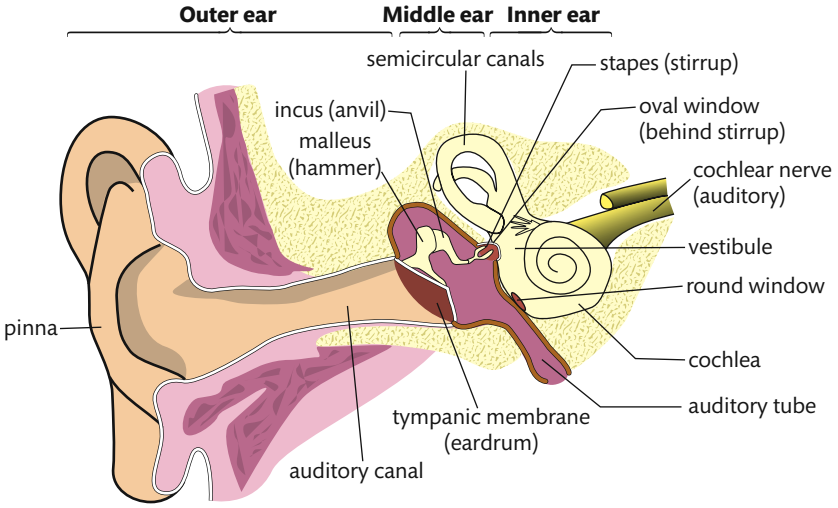


Figure 12.25 Anatomy of the human ear.

How sound is perceived by the ear

Sound waves are successive vibrations of air molecules caught by the outer ear. When they travel down the auditory canal, they cause the eardrum (tympanic membrane) to move back and forth slightly.

Structures in the middle ear transmit and amplify sound

- The bones of the middle ear, the malleus, incus, and stapes, receive vibrations from the tympanic membrane and multiply them approximately 20 times.
- The stapes strikes the oval window, causing it to vibrate.
- This vibration is passed to the fluid in the cochlea.
- The fluid in the cochlea causes special cells, called hair cells, to vibrate.
- The hair cells, which are mechanoreceptors, release a chemical neurotransmitter across a synapse to the sensory neurone of the auditory nerve.
- Vibrations are transformed into nerve impulses.
- The chemical message stimulates the sensory neurone.
- Impulses caused by sound perception are transmitted to the brain by the auditory nerve.
- The round window releases pressure so fluid in the cochlea can vibrate.

CHALLENGE YOURSELF

12 Here is another opportunity to learn the parts of one of your receptors, the ear. Again find a picture online, print it out, and label it. Label the parts from memory and then begin to learn the bullet points given above in order. Do you have a younger brother or sister who might be fascinated by you telling them the story of how we hear?

Sensory hairs of the cochlea detect sounds of specific length

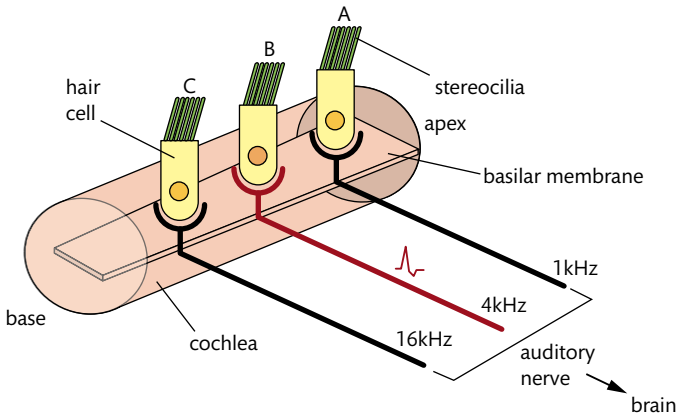


Figure 12.26 The stereocilia in the cochlea detect sounds of a specific wavelength. Hz measures the frequency of those sound waves. Hz is the SI symbol for hertz. A kHz is 1000 cycles per second of waves. Human hearing range is between 20 and 20 000 Hz.

The hair cells of the cochlea have stereocilia that stick out of the hair cells and detect sounds of a specific wavelength. As the stereocilia on the hair cells bend back and forth, an internal change in the hair cell itself is created. This change produces an electrical impulse that is carried to the auditory nerve.

Short, high-frequency waves produce high-pitched sounds, while long, low-frequency waves produce low-pitched sounds. The sound, which is sensed by the brain, is processed in the auditory area of the cerebral cortex. Hearing varies between people and changes with age. Hearing can also be affected by high-frequency noise. Listening to high-frequency sound for too long can damage the hair cells in the cochlea, and cochlear hair cells do not grow back. That is why many musicians wear protective ear devices when playing in concerts.

According to the *American Journal of Industrialized Medicine*, excessive noise is a global occupational health hazard resulting in noise-induced hearing loss (NIHL). Adult-onset hearing loss is the 15th most serious world heath problem.

The cochlea has more than 32 000 hair cells.

Here are some suggestions from a health professional on how to prevent hearing loss in teenagers.

You can enjoy listening to music yet avoiding harmful listening habits that can lead to permanent hearing loss, by following these steps.

- Switch to headphones: headphones isolate the background noise, so that you can hear the music with less increase in volume.
- Anything higher than 85 dB can cause damage.
- Listening for extended periods of time can impair hearing. Take breaks.
- Try the 60/60 rule: never turn your volume past 60% and only insert earphones for a maximum of 60 minutes per day.

Hair cells in the semicircular canals detect movement of the head

We have three semicircular canals in each inner ear. These semicircular canals control our equilibrium, and give our brain a three-dimensional report of our position. The canals contain fluid and hair cells. Movement of the fluid over the hair cells detects rotational movement of the head. The hair cells are sensory receptors that send messages to the vestibular nerve. This information, which is relayed to the brain, tells us our position. Are we upside down or falling backwards? We can maintain our balance in precarious positions because of the accuracy of the hair cells in the semicircular canals of our ears.

NATURE OF SCIENCE

Animal horns were the first hearing aid, used in the 13th century. Technology has developed many improvements over the years. In 1878, Francis Blake and David Edward Hughes discovered that carbon transmitters could amplify sound, which was a big advance over the horn! In 1920, the vacuum tube was invented. The Radioear was the name of one of the hearing aids that used a vacuum tube. In 1950, Bell Laboratories invented the transistor battery, which was small and a big improvement over the vacuum tube. Since 1967 the digital hearing aid has been the main type of hearing device. It is small and convenient to wear. However, we know that, using technology, scientists will develop future improvements to help those with hearing loss.

Use of cochlear implants in deaf patients

Cochlear implants are a product of medical technology that has improved the lives of people with severe to profound hearing loss when a hearing aid is not a solution. Cochlear implants convert sound into electrical signals that are sent directly to the brain. A cochlear implant works in the following manner.

- An external processor is worn behind the ear or attached to the hair.
- The microphone in the external processor picks up the sound signal.
- The external processor digitizes the sound and transfers the electrical signal to the implant, which has been surgically placed in the cochlea.
- The implant acts like a miniature computer, deciphering the digitized sound and transferring it into electrical signals.
- The auditory nerve picks up the electrical signals and sends a message to the brain.
- The brain interprets the signals as sound.

The following might be an interesting experiment to try with a class of students. Can most people identify the smells correctly? Is there a significant difference in males and females of the same age?

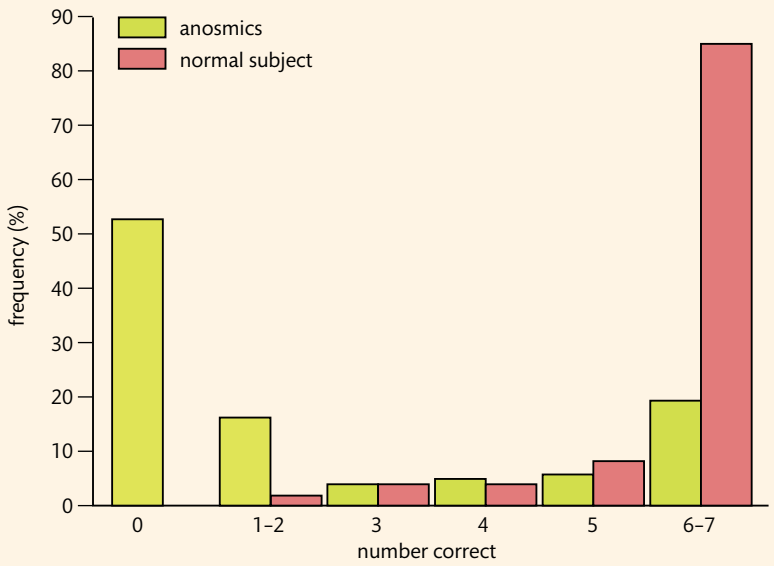


Figure 12.27 Anosmia. Purves et al. 2001

Anosmia is the inability to identify common smells. When subjects are presented with seven common smells (a test frequently used by neurologists), the vast majority of 'normal' individuals can identify all seven smells correctly. The smells used for the graph shown were baby powder, chocolate, cinnamon, coffee, mothballs, peanut butter, and soap. Some people, however, have difficulty identifying even these common smells. When individuals already identified as anosmics were presented with these seven smells, only a few could identify all of them (less than 15%), and more than half could not identify any of them.

To learn more about the evolutionary link between an animal's movement and its inner ear, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: A.3.

CHALLENGE YOURSELF

Look at Figure 12.28 and answer the following questions.

13 What % of smells can people between the ages of 20 and 40 identify? Give a range.

14 What % of smells can people between the ages of 50 and 70 identify? Give a range.

15 What name do we give to data that fall far above or far below the line of best fit?

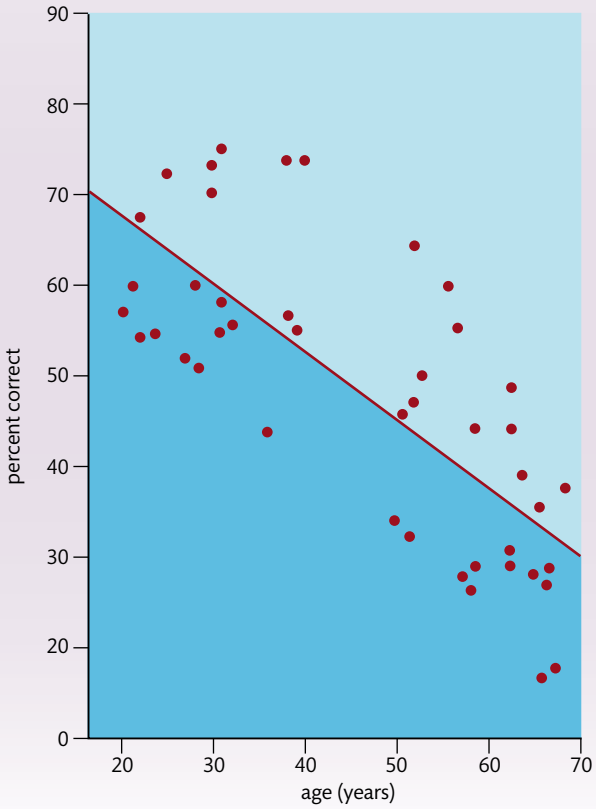


Figure 12.28 Normal decline in olfactory sensitivity with age. Purves et al. 2001

To learn more about sensory organs and colour blindness, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.3.

Exercises

10 Label a diagram of the retina and show the direction in which the light moves.

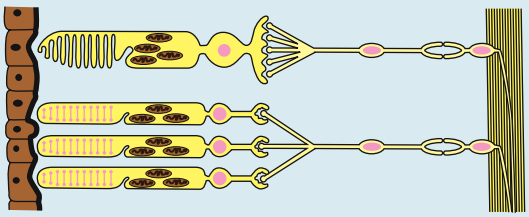


Figure 12.29 The structure of the retina.

11 Compare and contrast rods and cones.

12 Describe red–green colour blindness.

13 Outline the use of cochlear implants in deaf patients.

14 Explain how sound is perceived by the ear.

NATURE OF SCIENCE

Looking for patterns, trends, and discrepancies: laboratory experiments and field investigations helped in the understanding of different types of behaviour and learning.

A.4 Innate and learned behaviour

Understandings:

- Innate behaviour is inherited from parents and so develops independently of the environment.
- Autonomic and involuntary responses are referred to as reflexes.
- Reflex arcs comprise the neurones that mediate reflexes.
- Reflex conditioning involves forming new associations.
- Learned behaviour develops as a result of experience.
- Imprinting is learning at a particular life stage and is independent of the consequences of behaviour.
- Operant conditioning is a form of learning that consists of trial and error experiences.
- Learning is the acquisition of skill or knowledge.
- Memory is the process of encoding, storing, and accessing information.

Applications and skills:

- Application: Withdrawal reflex of the hand from a painful stimulus.
- Application: Pavlov's experiments into reflex conditioning in dogs.
- Application: The role of inheritance and learning in the development of birdsong.
- Skill: Analysis of data from invertebrate behaviour experiments in terms of the effect on chances of survival and reproduction.
- Skill: Drawing and labelling a diagram of a reflex arc for a pain withdrawal reflex.

Guidance

- *Drawing of reflex arc should include the receptor cell, sensory neurone, relay neurone, motor neurone, and effector.*

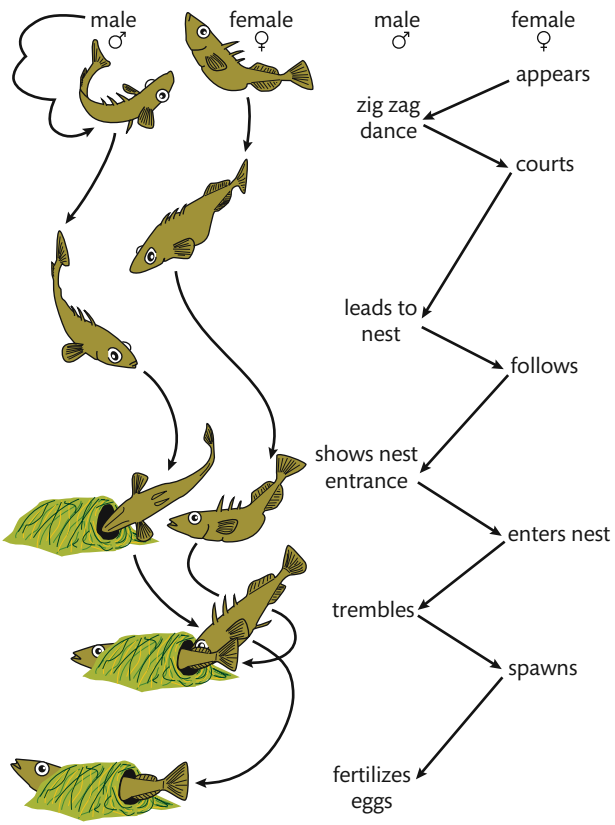


Figure 12.30 The courtship behaviour of the three-spined stickleback.

Innate behaviour

Innate behaviour is inherited from parents and so develops independently of environmental context. Innate behaviours are controlled by genes and inherited from parents. A spider spins a web correctly the very first time. No trial-and-error learning is taking place. A wasp builds a nest that is characteristic of its species. A termite builds a characteristic mound. Scientists familiar with insects can tell which species built a nest or mound by looking at its shape. These are genetically programmed behaviours, which ensure the survival of the animal. A simple version of song is innate in birds. Sucking behaviour is innate in human infants.

Some innate behaviours are performed in a certain order. A classic example of an innate sequence of behaviours is seen in the mating behaviours of the three-spined stickleback fish (see Figure 12.30). Mating begins with a male doing a zigzag dance when he sees a female. This dance attracts the female's attention. She follows the male as he leads her to the nest he has constructed in the bed of the river. He backs out of the nest and the female enters. He vibrates his body at the entrance to the nest and the female releases her eggs. She leaves the nest and the male enters. He releases his sperm, which fertilize the eggs. This behaviour is as specific to this species as the number of spines they have on their back.

Innate behaviour in invertebrates

Animals orient in different ways to their diverse environments. They survive better in some places than others. Food may be more plentiful in one area, better protection may be available in another area, humidity levels may be better in another. When studying simple invertebrate animals, innate behaviours can be measured as the animals respond to environmental stimuli. Two basic kinds of movement are seen in invertebrate animals: taxis and kinesis.

Taxis

A taxis (plural taxes) is a directed response to a stimulus. If the animal's body is directed towards the stimulus, we say it has a positive response. If the animal's body is directed away from the stimulus, we say it has a negative response. For example, if an animal moves towards light, it exhibits a positive phototaxis. If the animal moves away from light, it exhibits a negative phototaxis. Taxes are identified by the type of stimuli to which the organism is responding.

Chemotaxis is the response to chemicals in the environment. Organisms in water can move towards or away from food or other chemicals that are dissolved in their aquatic medium. When exploring chemotaxis, experiments can be performed that vary the pH, the concentration of dissolved drugs, food, or pesticides.

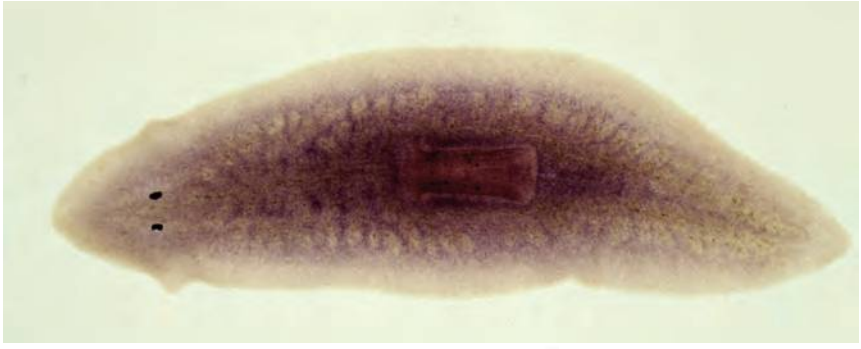
Phototaxis is the response to light. Experiments can be performed using different wavelengths of light, different light intensities and different types of bulb (ultraviolet, incandescent, or fluorescent).

Gravitaxis is the response to gravity. Methods can be devised to measure the response to gravity if organisms are put into a container that is then placed upside down. Placing organisms on a slow-spinning turntable may also disrupt the normal pull of gravity.

Rheotaxis is a response to water current. Do aquatic organisms move with or against the current?

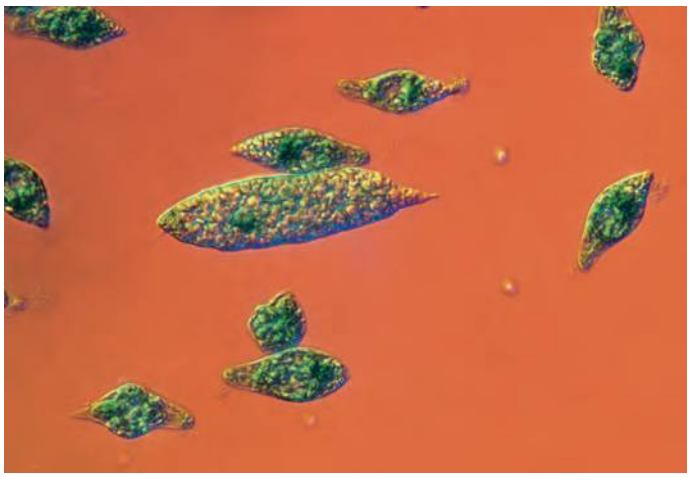
Thigmotaxis is a response to touch. It is interesting to see if an organism has a positive thigmotaxic response. Two invertebrates you can use to investigate taxes are *Planaria* and *Euglena*.

Planaria is a flatworm that lives in lakes and ponds. It is quite active and moves by contraction of muscle fibres in its body. It has a simple nervous system and at the anterior end are two eyespots that contain photoreceptors stimulated by light. Also in the anterior end are chemoreceptors that respond to certain chemicals. *Planaria* is negatively phototaxic, because it lives under leaves and rocks and hides for protection. It is positively chemotaxic to food that it likes to eat, such as raw liver (raw liver is similar to the dead fish in its natural habitat). Interesting studies to carry out can include *Planaria*'s response to different wavelengths of light or how fast it moves towards different food substances (measured as centimetres per minute).



This planarian is part of the group Turbellaria. You can see the two eyespots that are sensitive to light.

Euglena is single-celled protist (protoctist). It has a flagellum that propels it quickly through the water. It also has an eyespot at the anterior end that is stimulated by light. *Euglena* can make its own food by photosynthesis because it contains molecules of chlorophyll. It is positively phototaxic because it needs light to perform photosynthesis. *Euglena* can be tested to determine whether it responds to different wavelengths of light.



Euglena lives in ponds and puddles. This single-celled organism has a tail-like flagellum for locomotion. The cytoplasm is green because of the presence of a large number of photosynthetic chloroplasts that make food for the organism using sunlight.

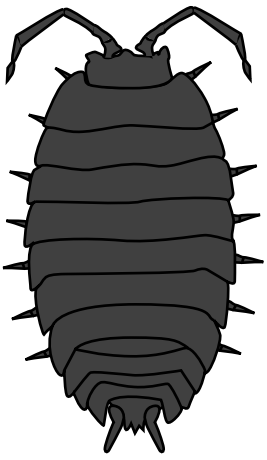
Kinesis

Kinesis is a movement in response to a non-directional stimulus, such as humidity. The rate of movement of the animal depends on the intensity of the stimulus, not its direction. It differs from taxis in that the animal does not move towards or away from the stimulus. If the animal is in an environment that is not suitable, it moves rapidly but randomly (with no direction) until it is in a more comfortable spot. If it is in its 'comfort zone', its movement slows down. Slow movement is likely to keep the organism in the environment that it prefers.

Orthokinesis is when an organism moves slowly or rapidly (changes speed) in response to a stimulus, but it does not move towards the stimulus.

Klinokinesis is when an organism turns slowly or rapidly in response to a stimulus, but it does not move towards the stimulus.

Isopods are terrestrial crustaceans that can be used to study kinesis. Even though they live on land, they breathe with gills and need moisture to breathe. Isopods live in damp places and die if exposed to dry conditions for a long period of time. Isopods show kinesis to humidity. When placed in a damp environment, they move slowly. When placed in a dry environment, they move quickly. Moving quickly makes it more likely that they will get out of the dry environment. Conversely, an isopod in a damp place will remain there because that place suits it. An isopod in a dry place may find a damp spot during its increased random movement. The minute it senses a damp environment, its random movements slow down. Two isopods used to study kinesis are the woodlice *Porcellio scaber* and *Armadillidium vulgare* (see Figure 12.31).



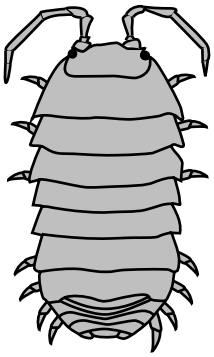


Figure 12.31 *Porcellio scaber* (left) and *Armadillidium vulgare* (right).

Experimental design

Follow these steps to design an experiment to investigate innate behaviours of an invertebrate.

1 Observe the organism of choice. Research the organism and formulate a research question, which must be specific. It must allow you to collect measurable data. Here is an example of a good research question: 'What is the effect of humidity on the distribution of the isopod *Porcellio scaber*?'

2 Describe a method for the collection of relevant data. Here is an example.

(a) Modify a pair of Petri dishes to make a choice chamber, in which the isopods are given an opportunity to be in humid or dry conditions. Set up one chamber with a drying agent ($CaCl_2$) and the other with wet towels (see Figure 12.32). Measure the humidity in each chamber with a Vernier probe.

(b) Place 10 individuals in each chamber.

(c) Count the number of individuals in each chamber every 5 minutes.

(d) Repeat this procedure so that you have data for 40 organisms.

(e) As a control, set up a pair of Petri dishes that have no difference in humidity. Repeat steps (b), (c), and (d).

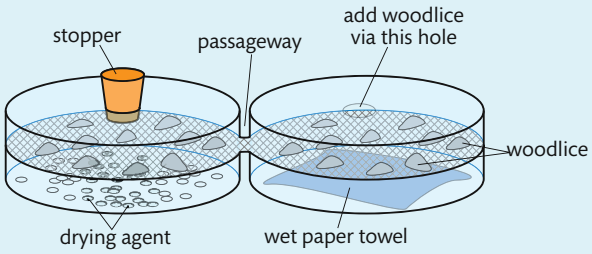


Figure 12.32 Apparatus for studying the orientation of isopods, set up to study humidity preference in constant light.

3 Design a method for control of the variables.

(a) Measure the light conditions in which the experiment is taking place with a Vernier probe. Make sure that the light conditions for the entire experiment remain constant. Isopods may respond to light as well, so the amount of light must be controlled.

(b) Measure the temperature conditions in which the experiment is taking place with a Vernier probe. Make sure the temperature conditions for the entire experiment remain constant.

(c) There must be an equal possibility for the isopods to travel to either chamber.

(d) The sizes of the chambers must be equal.

4 Record raw data, including units (minutes) and uncertainties (± 0.5 minutes). Make sure you write a title for each data table. Do not split a data table across two pages.

Time / min ±0.5	Chamber with desiccant (dry)	Chamber with wet towels (humid)
0	10	10
5	9	11
10	9	11
15	9	11
20	8	12
25	7	13
30	6	14
35	5	15
40	5	15
45	5	15

Table 12.8 Effect of humidity on the movement of isopods: trial 1

Table 12.9 Effect of humidity on the movement of isopods: trial 2

Time / min ±0.5	Chamber with desiccant (dry)	Chamber with wet towels (humid)
0	10	10
5	9	11
10	8	12
15	8	12
20	7	13
25	6	14
30	5	15
35	5	15
40	5	15
45	5	15

Table 12.10 Effect of humidity on the movement of isopods: control trial 1

Time / min ±0.5	Chamber empty	Chamber empty
0	10	10
5	9	11
10	9	11
15	9	11
20	9	11
25	9	11
30	10	10
35	10	10
40	10	10
45	10	10

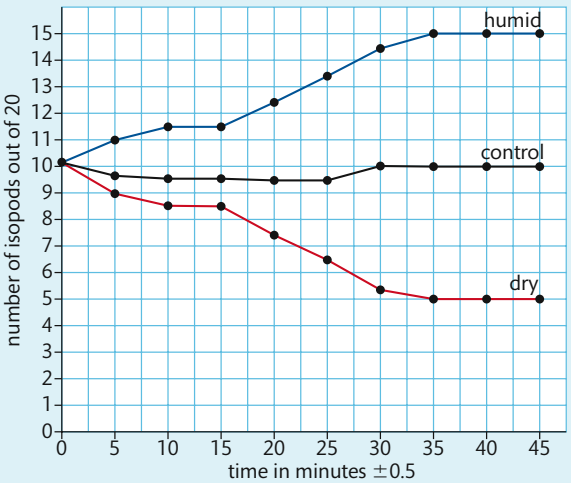


Figure 12.33 Graph of the mean values from two trials with isopods in humid and dry conditions.

Time / min ±0.5	Chamber empty	Chamber empty
0	10	10
5	10	10
10	8	12
15	8	12
20	8	12
25	9	11
30	9	11
35	10	10
40	10	10
45	10	10

Table 12.11 Effect of humidity on the movement of isopod: control trial 2

5 Process the raw data. Processing includes any mathematical manipulation of the data or graphing of manipulated data (graphing of raw data is not considered to be processing).

For example, determine the means of the numbers of isopods in humid and dry conditions after 45 minutes (±0.5) in the two trials, compared with the controls.

	Dry chamber	Humid chamber	Control chamber	Control chamber
Trial 1	5	15	10	10
Trial 2	5	15	10	10
Sums of two trials and controls	10	30	20	20
Means of two trials and controls	5	15	10	10

Table 12.12 Effect of humidity on the movement of isopods (means)

6 Graph the mean values from the two trials. For example, see Figure 12.33.

What do the invertebrate behaviour experiments tell us about survival and reproduction? First, we need to use statistical analyses to determine whether the differences are significant. For example, the chi-square statistical test can be used on these data to determine whether the differences between the data from the dry and the humid chambers are significantly different compared with the data from the controls. Second, we can draw conclusions based on the statistical analysis of the data. The behaviour of the isopods is to move randomly and quickly in a dry environment until they finally come to rest in the humid environment. Humidity is important to the survival of isopods and their ability to reproduce. The outer covering of isopods (exoskeleton) lacks a waterproof waxy cuticle (as found in many land-dwelling groups), so they are highly subject to desiccation (drying out). Quick random movements enable isopods to find themselves in a humid environment. This ensures survival and enhances the ability to reproduce. Natural selection favours isopods that show this response.

TOK

As you can see from the isopod experiment, scientific method uses inductive reasoning to go from the particular to the general. For example, we say that when all metals are heated they expand. As far as we know, all metals that have been heated, do expand. However, we are now moving from the observed to the unobserved. We have not measured all the metals there are in the world to see if they expand. This raises the practical problem of how many data points we need in an experiment in order to make a valid generalization. We have a tendency to use insufficient evidence and jump to conclusions. Discuss the limits of scientific knowledge. Does scientific knowledge change with more experimentation? Can you give some current examples?

Worker honeybees are female bees that have their ovipositor modified into a stinger. The bee stings its victim with its barbed stinger, then flies away, leaving the stinger in the victim. The stinger continues to pump venom, and to embed itself deeper into the victim's skin.

Reflexes

Have you ever been stung by a bee? The first thing you notice is the pain. The sting is the stimulus. Fortunately, we have receptors for pain. The receptor notifies us of the pain. Your immediate response to the sting is a pain withdrawal reflex. The pain reflex involves a series of nerves that run from your arm to your spinal cord, and back to your arm muscle. This causes you to brush off the bee before you can even think about it. Reflexes take place so quickly because they do not have to travel up to your brain to be processed. You do not control reflexes with your brain. Reflexes are controlled by the autonomic nervous system. The autonomic nervous system also controls digestion and blood pressure.

Reflex arc

A reflex arc is composed of a receptor cell, a sensory neurone, a relay neurone in the spinal cord, and a motor neurone, which carries the message to the effector (muscle). Usually, reflexes are protective. For example, the pupil reflex protects our eyes from excessive light that can damage the eye. The blink reflex also protects the eye from damage. Sneezing is a reflex that clears the airways of irritations. The pain reflex described below is something we have all experienced.

Pain reflex

- Receptor cells receive the stimulus. For example, pain receptors receive the stimulus of heat, pressure, or chemicals produced by injured tissues. If you prick your finger on a needle, the pain receptors respond to the injured tissue.
- The receptors detect a stimulus and generate a nerve impulse in the sensory neurones (see Figure 12.34).
- The sensory neurones carry the impulse towards the spinal cord.
- The axon of the sensory neurone enters the spinal cord and sends a chemical message across a synapse to a relay neurone.
- The relay neurone synapses with the motor neurone and transfers the impulse chemically across the synapse.
- The motor neurone carries the impulse to an effector.
- An effector is an organ that performs the response. In this case, the effector is a muscle that contracts and pulls the finger away from the needle.

Reflex conditioning

Reflex (classical) conditioning can be used to modify a reflex response. In reflex conditioning experiments, the subject responds to a stimulus in a new way. For example, in humans, blinking is a reflex response. If you wave your hand suddenly in front of someone's face, he or she will automatically blink. The waved hand is called the unconditioned stimulus (UCS) because it unconditionally stimulates the eye blink response. The eye blink is called the unconditioned response (UCR). After training, it is possible to elicit the reflex response (eye blink) with a new and neutral stimulus (NS). First, a neutral stimulus (e.g. a musical note) is introduced to someone. That person probably does not blink. Next the person is given a period of training: the musical note is sounded immediately before a hand is waved in front of his or her eye. Eventually the person responds with an eye blink to just the musical note. After this has occurred,

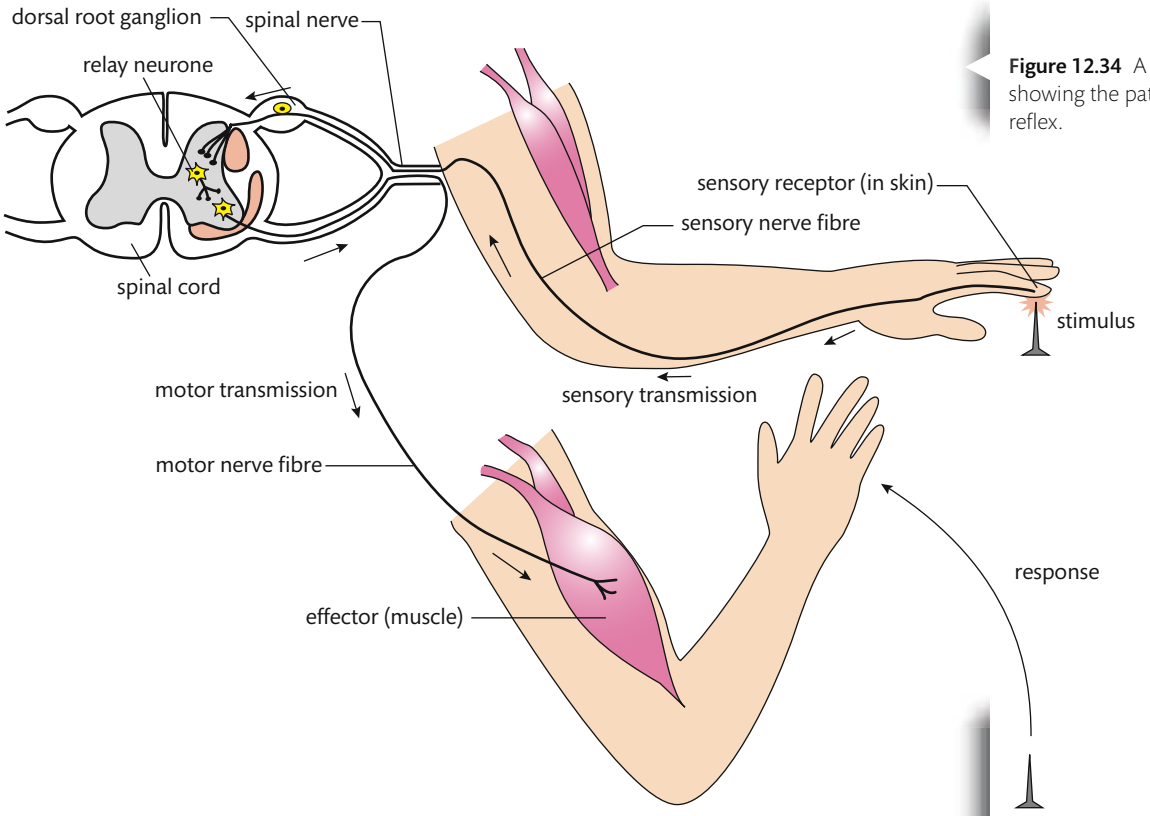


Figure 12.34 A reflex arc showing the path of a spinal reflex.

the musical note is called the conditioned stimulus (CS) and the eye blink in response to the musical note is called the conditioned response (CR). The person is now responding to a musical note in a new way.

The Russian physiologist Ivan Pavlov designed experiments to illustrate reflex (classical) conditioning. His subjects were dogs. Salivation in dogs is a reflex response to the presence of food in the mouth. The UCS of food elicits the UCR of salivation. The NS that Pavlov used was the ringing of a bell (see Figure 12.35). He rang the bell just before the dogs tasted their food. After training, he could ring the bell (CS) and the dogs would salivate (CR). The dogs had learned to salivate to the NS alone.

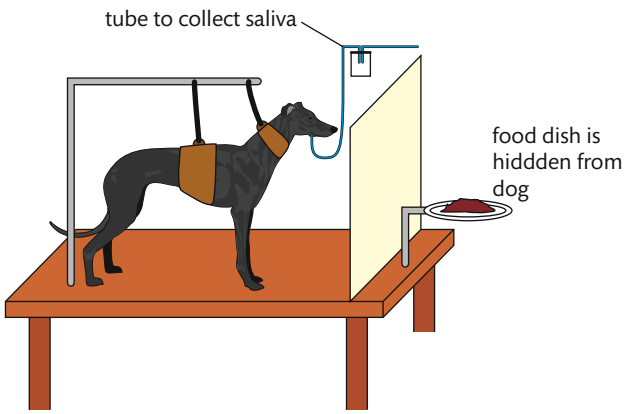


Figure 12.35 Pavlov's experimental setup.

CHALLENGE YOURSELF

16 Practise drawing and labelling a reflex arc for the pain withdrawal reflex.

TOK

Can the Pavlov theory of conditioning be applied to different examples of learning? Consider this example of learning that could take place at the dentist. If drilling into your tooth causes you pain, the drilling can be considered the UCS, and the pain the UCR. Now when you return to the dentist's office and you hear the sound of the drill (CS) in the next room, you may feel the pain return to your tooth (CR). Can you think of other examples of learning to which you can apply conditioned and unconditioned responses?

Learned behaviour

Learned behaviour is not genetically programmed, it develops as a result of experience. You learned to read a book, how to ride a bike, how to tie your shoes. All of these activities result in new knowledge that did not previously exist or a new skill that you did not originally possess. Learned behaviour can be defined as the process of gaining new knowledge or skills. You may know how to read some things when you are 6 years old, but you will improve that skill with more practice and schooling.

Can we apply the same principles of learning to animals? Learning can only be measured by performance. Learning can be explained as a change in performance that is stored in the nervous system as memory. For example, a rat can learn that pressing a pedal releases food. The rat originally pressed the pedal by accident during exploration of its cage. After the pellet of food was released over and over again, the rat learned to associate the food with the pedal. Pushing the pedal for food then becomes a deliberate act. This is a performance, which indicates learning. However, behavioural output is not always easily seen, which is why learning is sometimes difficult to measure.

Table 12.13 tables summarizes innate and learned behaviour.

Table 12.13 A comparison of innate and learned behaviour

Innate behaviour	Learned behaviour
Develops independently of the environmental context	Is dependent on the environmental context of the animal for development
Controlled by genes	Not controlled by genes
Inherited from parents	Not inherited from parents
Developed by natural selection	Develops as a result of experience
Increases the chance of survival and reproduction	May or may not increase chance of survival and reproduction

Imprinting

Konrad Lorenz followed by young goslings that have imprinted him as their mother.

An interesting story of imprinting involves the first ethologist (someone who studies animal behaviour under natural conditions), Konrad Lorenz, and how he ended up with baby graylag geese (goslings) following him around! Why would geese become attached to a scientist? Lorenz noticed that goslings always follow their mother everywhere in the first stages of their life. He wondered how and when that learning occurred. After many observations, he realized that imprinting was initiated during a critical period: 13–16 hours after hatching. Then he wondered whether the goslings imprint on any moving object. Lorenz hatched some goslings in an incubator so that the first moveable object that they saw was Lorenz. The goslings imprinted on him and followed Lorenz around the farm. In a follow-up experiment, he found that the goslings would imprint on a box attached to a model train. Amazingly, the goslings would follow the box on the model train around the track.

Lorenz came to the understanding that imprinting happens at a particular stage in life. It is a rapid learning process by which a young animal develops an attraction and recognition of another moving object, usually the mother. However, because an animal can imprint on an inanimate object, like a box on a model train, the consequence of the behaviour is not something that reinforces it. The behaviour occurs regardless of the consequences.

The movie *Winged Migration* is a story of how birds were trained using imprinting to fly along with ultralight airplanes.

Parent recognition is called filial imprinting. This type imprinting is strongest in chicks of species that do not stay in a nest after hatching, such as ducks, geese, and grouse. These young must be able to follow their parent so that they can find their own food. Young birds that stay in a nest are fed by their parents.

Operant conditioning is a form of learning

B. F. Skinner was a scientist who worked in the 1930s. He developed an apparatus called a Skinner box. Inside the Skinner box he would place a rat or pigeon. The box was small and the animal had not much to do except explore the cage. Eventually, it would accidentally touch the lever that was in the box, which would deliver a pellet of food. By depriving the animal of food, it would gradually learn that pressing the lever would release a pellet of food into the feeding dish. The process of associating food with the lever may take a long time, but eventually the animal learns to manipulate its environment. The following guidelines describe this type of learning, called operant conditioning.

- The original behaviour is performed spontaneously during exploration of the environment.
- The experimenter wishes to change the likelihood of this behaviour being performed. This behaviour is called an operant.
- The Skinner box makes it easy to study operant conditioning.
- There is no interference from the experimenter.
- In operant conditioning, the animal brings about a change in its environment by performing a particular pattern of behaviour. In the example with the pigeon or rat, the change in the environment is food being released, and the pattern is repeatedly pressing the lever.

A rat in a Skinner box.

Young male sparrows sing a crude species-specific song that is inherited. As they mature, they learn to sing a better song by listening to other adult males sing.

Learning is the acquisition of skill or knowledge

What skills have you learned recently? Have you learned how to play a new game, or play an instrument, or speak a new language? We are acquiring new skills and knowledge daily. What skills must animals learn to survive and reproduce effectively? Birdsong is a good example of the acquisition of a skill or knowledge by an animal.

Are you able to tell the difference between birdsongs? Each species of bird has a species-specific song that is inherited. Birds of one species have a varied song, just as we have variations in the colour of our eyes. However, birds can also learn to improve the song they have inherited. Thus birdsong has both inherited and learned components.

Birds are able to sing because of their vocal organ, called the syrinx. The syrinx is a bony structure at the bottom of their trachea (windpipe). In humans, the larynx (voice box) is at the top of the trachea. The bird forces air past a membrane in the syrinx that vibrates and results in sound. Birds control the pitch by altering the tension in the membranes of the syrinx. They control the volume of the song by altering the flow of air.

Birdsong is a well-studied example of animal behaviour. Singing is an important business for the male bird. He attracts a mate with his song and deters male rivals. Generally females do not sing. Studies of song learning have shown that birds hatch with what is called a 'crude template'. Evidence for this template has been shown with experimental data: if birds are kept in a laboratory and denied any auditory stimulation, they produce a very crude song. This crude song is species-specific. The crude song of a warbler can be distinguished from the crude song of a sparrow. With an acoustical spectroscope, it is possible to measure the difference accurately. Such data provide evidence that the template is inherited. All of the next steps of birdsong development are learned (see Figure 12.36).

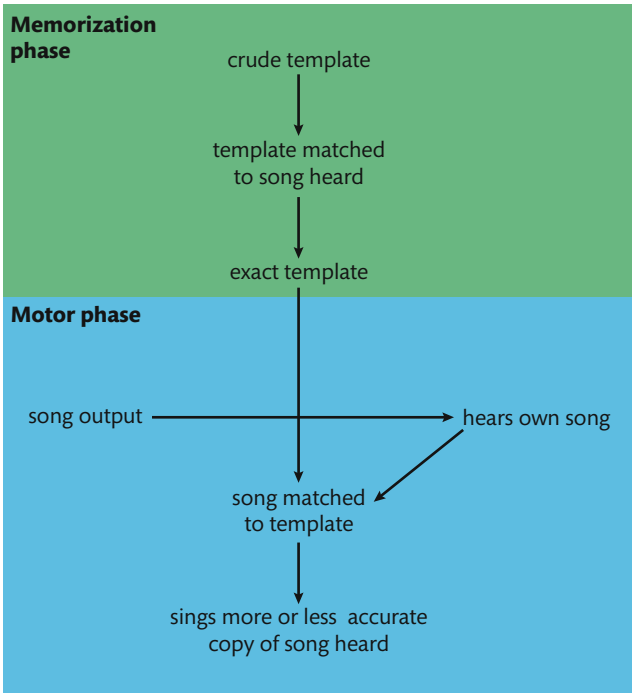


Figure 12.36 A crude template of birdsong is inherited, but the development of a mature adult song is learned.

After hatching, there is a memorization phase. In this phase, the bird is silent but listening to the song of an adult of his species. The hatchling is modifying the inherited template.

As he is listening, he is attempting to match his template to the full adult song. It is a type of memorization. This memorization phase is over at about 100 days of age. If a male bird does not hear the adult song within 100 days, he will not modify the template that he has inherited. These first 100 days are called the sensitive period.

The second phase is a motor phase, in which the young bird practises singing the song that he has heard. He hears himself singing and begins to shape his song to match what he has heard from an adult, usually his father. The bird must hear his own song in order to sing an accurate adult song. Experiments have been done that show that if a bird is deafened for 100 days, he will only sing the crude template of the song. As he becomes sexually mature, his song will become perfected and he will begin to search for a mate.

The crude template is a good example of innate learning, while the adult song is an example of how learned behaviour can help an animal acquire new skills.

The first global survey of bird diversity will tell us which species are most vulnerable to extinction. If a species is extinct we will never hear its song again. An international team is trying to determine the range of each bird species on a global scale. Those birds with a smaller range are at greater risk of extinction. The group will collect the global data that are important to understanding how conservationists can make a difference to bird survival.

Memory

How much did you remember about genetics when you took a biology exam? As learning can only be measured by performance, the success of a learned experience depends on recall. Could you do the questions about sex-linked traits, like red–green colour blindness? If you did remember how to answer those questions, then you had learned the relevant information and stored it so you could recall it later. You used the following processes needed for memory: encoding, storage and accessing.

Encoding

During encoding, the brain processes information that it receives from the senses so that it can be remembered. The following are some types of encoding carried out by the brain.

- Visual encoding: converting information into mental pictures.
- Elaborative encoding: relating new information to old knowledge already stored in the memory.
- Acoustic encoding: encoding of sound, such as spoken language.
- Sensation encoding: encoding of sensations such as touch, smells, and tastes.
- Semantic encoding: remembering sensory input in a context. For example, using a mnemonic device such as ROYGBIV as a strategy to remember the colours of the light spectrum in order (red, orange, yellow, green, blue, indigo, and violet).

Greek philosophers used mnemonic devices a lot and are repeatedly referred to by Plato and Aristotle. Quite a lot of factual information in biology can be remembered more easily if you make up a mnemonic device.

Storage

The ability to store information allows us to maintain the knowledge gained for a certain period of time. Storage occurs at the level of the neurone. Neurones synapse with each other using molecular signals. With increased numbers of transmitted signals, the strength of the synaptic connection also increases. This is why practice makes perfect. If you sing a song over and over again, repeating the signals between the neurones makes a better memory of it. Practise singing the song enough and you will sing it perfectly. You can see how new memories are created by education and experience.

If something is forgotten, it is usually because the synaptic connection has become weakened. If you know how to answer genetics problems about sex-linked traits for the biology exam this year, you might not remember how to do them next year without some more revision.

Accessing

Accessing is the retrieval of stored memories. There are two types of memory: short-term memory (STM) and long-term memory (LTM).

- STM holds a small amount of information for a short period of time. It holds on to items we are actively thinking about. Information stored here is retrieved in sequence.
- LTM information is stored over a long period of time. LTM involves a physical change in the neural network of the brain. With repeated use, the circuits of the brain are altered and strengthened.

The two main methods of accessing memories are recognition and recall.

- Recognition is the association of a physical object or event with something already experienced. It involves comparing present information with memory. If you see a face in the crowd and remember who that is, that is recognition.
- Recall involves remembering a fact, object, or event that is not currently present. Actively reconstructing the memory requires the activation of all the neurones involved in the memory. This is much more complicated than recognition. Recognition simply requires a decision about whether or not something has been encountered before.

A multiple-choice test is easier than an essay test because you can recognize something you have seen before within limited choices. An essay question is more difficult because it requires you to recall all of the information from memory. One reason why revising before an exam is beneficial is that 'recall' drags up a memory from your LTM and puts it back in your STM (or working memory). Eventually, what you are revising is stored back in your LTM, where it is now stronger.

To learn more about memory, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.4.

Exercises

15 Draw and label a reflex arc for the pain withdrawal reflex.
16 Outline a lab experiment using reflex conditioning.
17 Compare and contrast innate and learned behaviour.
18 Explain the processes involved in memory.

A.5 Neuropharmacology

Understandings:

- Some neurotransmitters excite nerve impulses in postsynaptic neurones, and others inhibit them.
- Nerve impulses are initiated or inhibited in postsynaptic neurones as a result of summation of all excitatory and inhibitory neurotransmitters received from presynaptic neurones.
- Many different slow-acting neurotransmitters modulate fast synaptic transmission in the brain.
- Memory and learning involve changes in neurones caused by slow-acting neurotransmitters.
- Psychoactive drugs affect the brain by either increasing or decreasing postsynaptic transmission.
- Anaesthetics act by interfering with neural transmission between areas of sensory perception and the CNS.
- Stimulant drugs mimic the stimulation provided by the synaptic nervous system.
- Addiction can be affected by genetic predisposition, social environment, and dopamine secretion.

Applications and skills:

- Application: Effects on the nervous system of two stimulants and two sedatives.
- Application: The effect of anaesthetics on awareness.
- Application: Endorphins can act as painkillers.
- Skill: Evaluation of data showing the impact of MDMA (ecstasy) on serotonin and dopamine metabolism in the brain.

Guidance

- *Examples of stimulants are nicotine, cocaine, or amphetamines.*
- *Examples of sedatives are benzodiazepines, alcohol, or tetrahydrocannabinol (THC).*

NATURE OF SCIENCE

Assessing risks associated with scientific research: patient advocates will often press for the speeding up of drug approval processes, encouraging more tolerance of risk.

Synaptic transmission

You may remember that neurones communicate with each other chemically across a space called a synapse. On one side of the synapse is the presynaptic membrane of the sending neurone, and the other side of the synapse is the postsynaptic membrane of the receiving neurone. When you send your friend an email, you are like the presynaptic membrane and your friend is like the postsynaptic membrane. The molecule that moves across the space (synaptic cleft) between the two membranes is called a neurotransmitter. Your email is like the neurotransmitter, moving from the presynaptic membrane to the postsynaptic membrane. At the postsynaptic membrane, there is a receptor molecule, which is like the inbox of your email. A specific neurotransmitter is received by a specific receptor, which is similar to how you receive an email with your address on it.

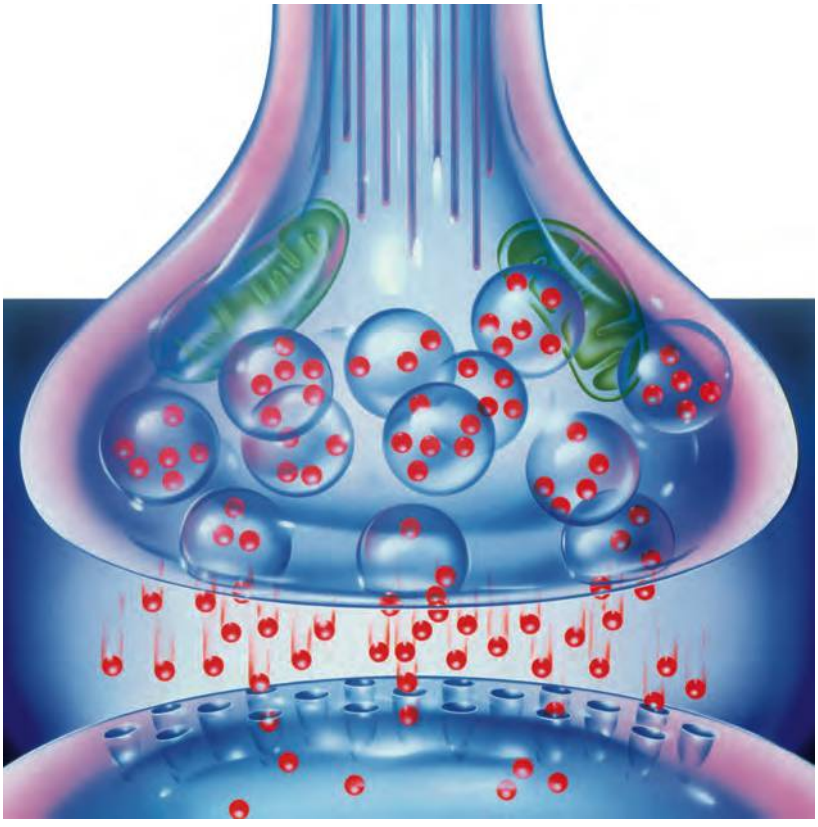


Figure 12.37 At the synapse, some presynaptic neurones excite postsynaptic neurones and others inhibit postsynaptic transmission. Note that the neurotransmitter is received by a receptor but does not enter the postsynaptic neurone.

Some neurotransmitters are excitatory and stimulate the next neurone to forward the message. The way they do this is to increase the permeability of the postsynaptic membrane to positive ions, making it easier for positive ions to move in. Some neurotransmitters are inhibitory. They cause positive ions to move out of a postsynaptic neurone. The movement of positive ions back into the synaptic cleft chemically depresses the postsynaptic neurone and makes it much harder to excite.

From this description you can see that some presynaptic neurones excite postsynaptic neurones and others inhibit postsynaptic neurones.

Interactions at the synapse

What interaction occurs between excitatory and inhibitory presynaptic neurones acting at the synapse?

The impulse that moves down the presynaptic neurone is called the action potential. As the action potential reaches the axon bulb, calcium ions (Ca^{2+}) rush into the end of the neurone. This causes vesicles containing neurotransmitters to fuse with the presynaptic membrane. As the vesicles fuse with the presynaptic membrane, they release the neurotransmitters into the synaptic cleft (see Figure 12.38).

Once the neurotransmitter is in the synaptic cleft, it binds to specific receptors on the postsynaptic membrane. The receptors are like gates that let ions enter or leave when the neurotransmitter binds to them.

Excitatory neurotransmitters

Acetylcholine is an example of a neurotransmitter that is excitatory. Excitatory neurotransmitters generate an action potential. Excitatory neurotransmitters increase the permeability of the postsynaptic membrane to positive ions. This causes positive sodium ions (Na^+) that are in the synaptic cleft to diffuse into the postsynaptic neurone. The postsynaptic neurone is depolarized locally (just in that area) by the influx of positive sodium ions. During depolarization, the inside of the neurone develops a net positive charge compared with the outside. Depolarization is the way the impulse is carried along the neurone. The neurone is locally depolarized and the depolarization continues as sodium ions diffuse to the next area of the neurone. In this fashion, the impulse is conducted along the neurone from one adjacent area to the next, just like a wave.

An action potential is formed as the membrane depolarization is raised above the threshold. This means that the impulse is being carried along the nerve. If the threshold is not met, the neurone does not carry the impulse to the next neurone.

Inhibitory neurotransmitters

GABA (gamma-aminobutyric acid) is an example of an inhibitory neurotransmitter. Inhibitory neurotransmitters inhibit action potentials. Inhibitory neurotransmitters cause hyperpolarization of the neurone (the inside of the neurone becomes more negative), making it even more difficult for an action potential to be generated.

The inhibitory neurotransmitter binds to its specific receptor. This causes negatively charged chloride ions (Cl^-) to move across the postsynaptic membrane into the postsynaptic neurone, or it can cause positively charged potassium ions (K^+) to move

This is a useful trick for remembering the details of hyperpolarization: when the 'kat' (K^+) is 'hyper' (hyperpolarized), we put her outside. (K^+ moves out of the neurone.)

out of the postsynaptic neurone. This movement of Cl^- into the neurone or K^+ out of the neurone is what causes the hyperpolarization.

Summation

A postsynaptic neurone is on the receiving end of many excitatory and inhibitory stimuli. The postsynaptic neurone sums up the signals. If the sum of the signals is inhibitory, then the impulse is not carried forward. If the sum of the signals is excitatory, then the impulse is carried forward. This is the interaction that takes place between the activities of the excitatory and inhibitory neurones at the synapses. The summation of the messages is the way that decisions are made by the central nervous system (CNS).

Modulation of synaptic transmission

Slow versus fast neurotransmitters

Remember that a neurotransmitter is a chemical that binds to a receptor, so is a 'doorway' to the neurone. It does not go 'in' the door. It has an effect on the postsynaptic neurone that causes ion gates to open. The flow of ions causes either depolarization (a continuation of the nerve impulse) or hyperpolarization (a termination of the nerve impulse).

Two types of neurotransmitters (NTs) are found in the brain:

- fast-acting NTs, which have an effect on the target cell within 1 millisecond of binding to a receptor
- slow-acting NTs, which have an effect on the target cell in hundreds of milliseconds or can take up to a minute.

The slow-acting NTs act on a second messenger molecule. The second messenger molecule then directly affects the target cell. This two-step process is more time consuming.

Look at the diagram of synaptic transmission (Figure 12.38). You can see that Na^+ is moving through a gate in the postsynaptic membrane. This gate is called an ion channel because ions like Na^+ are moving back and forth across the postsynaptic membrane. No second messenger is needed, thus the speed is very fast.

Excitatory neurotransmitters:

- **increase the permeability of the postsynaptic neurone to Na^+**
- **causing Na^+ ions to diffuse in**
- **so the neurone is depolarized**
- **so the impulse is carried forward.**

Inhibitory neurotransmitters:

- **make the inside of the neurone more negative**
- **causing Cl^- to move in or K^+ to move out**
- **so the neurone is hyperpolarized**
- **so the impulse is inhibited.**

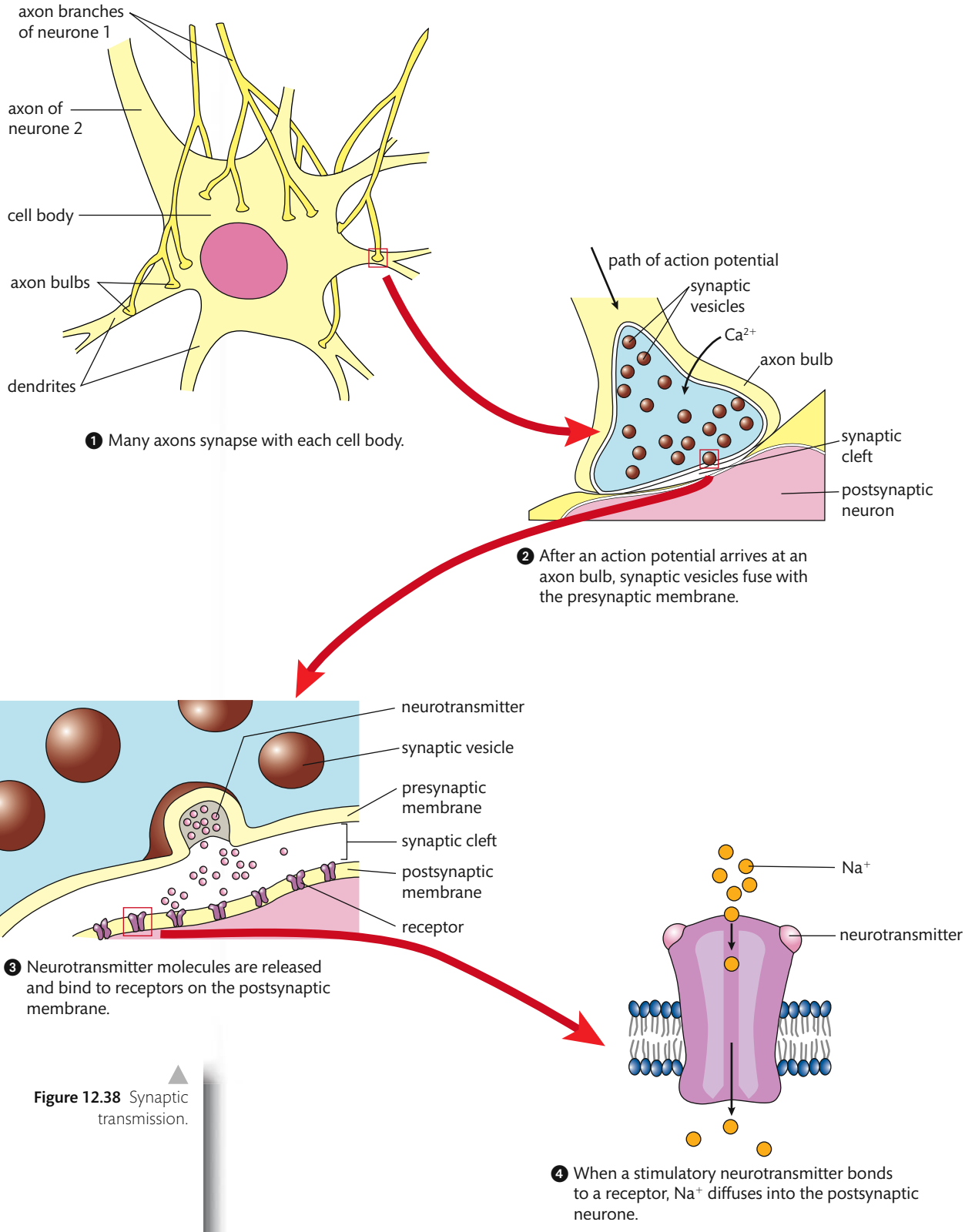


Figure 12.38 Synaptic transmission.

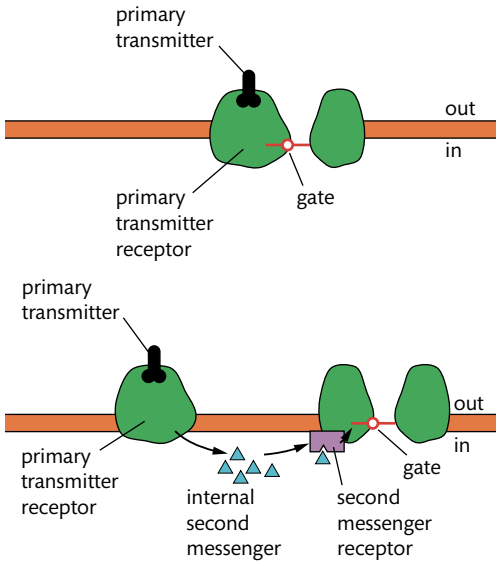


Figure 12.39 Fast and slow transmission at the postsynaptic membrane. Adapted from Hille 2001

Look at the diagram that compares fast-acting and slow-acting transmission in the brain (Figure 12.39).

The top diagram shows a fast-acting transmission. It shows a primary transmitter (neurotransmitter) that moves across the synapse and finds a primary receptor on the postsynaptic neurone. This opens the ion channel gate.

The bottom diagram shows a slow-acting transmission. Notice that an internal second messenger molecule is needed to open the ion gate; this is therefore a slower method. In fact, it can be 10–30 times slower because there are many biochemical steps between the internal second messenger and the second messenger receptor.

Examples of slow-acting neurotransmitters are:

- dopamine
- serotonin
- acetylcholine.

Slow-acting neurotransmitters modulate fast-acting neurotransmitters

Slow-acting neurotransmitters are sometimes called neuromodulators. They are released into the cerebrospinal fluid and modulate the fast-acting neurotransmitters in the brain. At least 100 compounds can be labelled as slow-acting neurotransmitters. These compounds can modulate fast-acting transmission in two ways.

- Slow-acting NTs can regulate the efficiency of neurotransmitter release from the presynaptic neurone (the sender).
- Slow-acting NTs can regulate the efficiency of the postsynaptic neurone (the receiver).

There are about 100 billion nerve cells in the brain and each of them communicates with about 1000 others.

Memory and learning

Learning

Why are we concerned about how slow-acting neurotransmitters affect fast-acting neurotransmitters in the brain? They are important because they affect our ability to learn and remember. The action of slow-acting neurotransmitters on neurones is

what allows the brain to have the functions of 'learning' and 'memory'. Scientists have investigated memory and learning at a cellular level by identifying specific molecular activities in certain nerve cells. Eric Kandel is a neuroscientist who began by studying the nerve cells of a model animal, the giant marine snail *Aplysia*. He found that by giving a puff of serotonin, a slow-acting neurotransmitter, to *Aplysia* the following would occur.

- A puff of serotonin (a slow-acting NT) acts on a presynaptic neurone.
- This causes an influx of calcium ions (Ca^{2+}) into the presynaptic neurone
- The increase in Ca^{2+} causes the production of cyclic adenosine monophosphate (cAMP), which is a second messenger.
- cAMP activates another molecule, protein kinase (PKA).
- PKA enhances the release of the NT from the presynaptic neurone into the synapse.
- The result is short-term learning in the gill withdrawal reflex of *Aplysia* (see Figure 12.40).

If the siphon of the snail is stimulated once, the gill is retracted. A second very slight stimulation caused the same strong retraction. Thus, some learning takes place. If four or five stimulations are given, the memory lasts several days. *Aplysia* learns to retract its gill when its siphon is stimulated as a protective device.

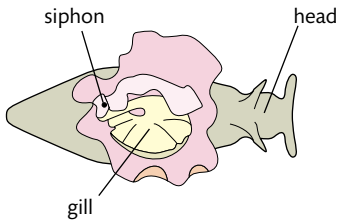


Figure 12.40 The marine snail *Aplysia* exhibits a gill-withdrawal reflex when its siphon is stimulated. http://www.nobelprize.org/nobel_prizes/medicine/laureates/2000/press.html

Isn't it amazing that the behaviour of an animal has a basis in the chemical activity of a nerve cell? The biochemical cascade mentioned above increases neurotransmitters in the synapse and strengthens short-term memory. As you can see, this process uses cAMP, which is a second messenger.

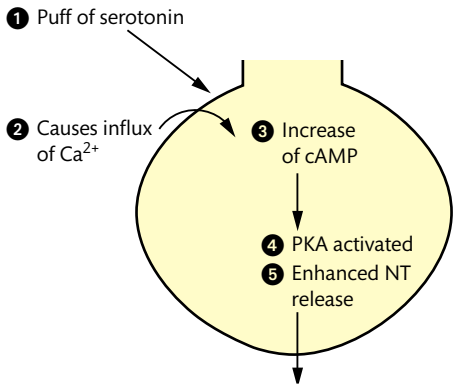


Figure 12.41 The effect of a slow-acting neurotransmitter on a presynaptic neurone.

Memory

Eric Kandel and his co-workers continued experiments with *Aplysia* and discovered how the long-term process (memory) differs from the short-term process (learning). The long-term process requires the synthesis of proteins. The synthesis of proteins necessitates the activation of genes in the nucleus of the neurone. The proteins change the form and function of the synapse, and this results in memory.

For a long-term memory to be created, a stronger, more long-lasting stimulus is needed. This time five puffs of serotonin were given to the *Aplysia*. Use Figure 12.42 along with the bulleted points below it to see the effect of extra serotonin in the synapse.

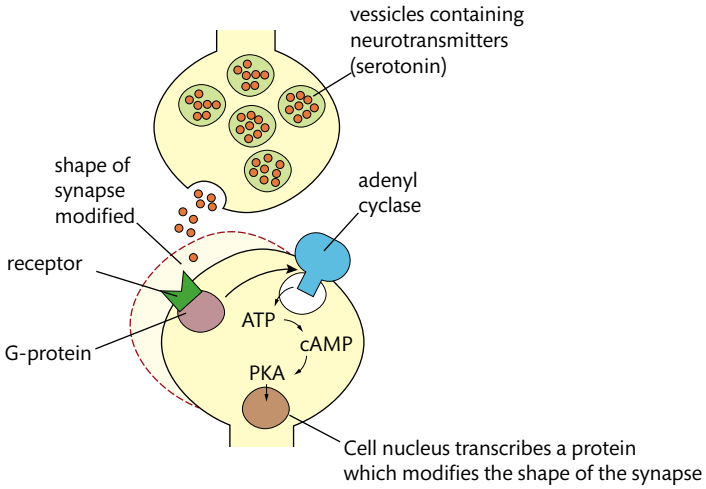


Figure 12.42 The effect of slow-acting neurotransmitters on the postsynaptic neurone.

- Five puffs of serotonin (slow-acting NT) act to increase the level of serotonin in the synapse.
- Serotonin in the synapse is received by a receptor on the postsynaptic membrane.
- Attached to this receptor is a G-protein.
- G-protein is activated and stimulates the adenyl cyclase enzyme that is also embedded in the cell membrane of the postsynaptic neurone.
- Activated adenyl cyclase facilitates the change of ATP molecules located in the cell to cAMP molecules.
- This causes high levels of cAMP (second messenger) to be produced.
- In turn, this activates PKA.
- This signal reaches the nucleus and activates transcription. With transcription, the genes make new proteins.
- Proteins travel out of the nucleus and modify the shape of the synapse. This can create a long-lasting change in synaptic function, including new connections.

As you can see, the activation of genes caused by extra serotonin in the synapse changes the form and function of the synapse, resulting in memory.

Psychoactive drugs affect the brain and personality

To understand fully how drugs affect the brain and personality, we must have an understanding of the two main neurotransmitters: acetylcholine and noradrenaline.

Cholinergic versus adrenergic synapses

Acetylcholine is released by all motor neurones and activates skeletal muscle. It travels across the synapse and depolarizes the postsynaptic membrane. However, if it remained in the synapse, the postsynaptic membrane would go on firing indefinitely. To prevent this, an enzyme called acetylcholinesterase breaks down acetylcholine in the synapse. Acetylcholine is involved in the parasympathetic nervous system. This means it causes relaxation rather than flight.

NATURE OF SCIENCE

From the 1930s to the 1960s, there was a big debate about whether communication between nerve cells was electrical or chemical. When Paul Greengard first presented the idea that biochemical pathways were at the root of learning and memory, the scientific community met his ideas with much scepticism. Do you know how the ideas of Galileo were first received? How does scientific knowledge progress?

Synapses using acetylcholine are called cholinergic synapses. Nicotine stimulates transmission in cholinergic synapses, which is why it has a calming effect on the body and personality. People addicted to nicotine become very agitated if they cannot have a cigarette.

Noradrenaline depolarizes the postsynaptic neurone; it is involved in the sympathetic system. This means that it causes a 'fight or flight' reaction. Synapses using noradrenaline are called adrenergic synapses. Cocaine and amphetamines stimulate adrenergic synapses. Cocaine and amphetamines both cause increased alertness, energy, and euphoria.

Table 12.14 compares cholinergic and adrenergic synapses.

The slow-acting transmission system studied the most intensively by Paul Greengard and his group was the dopamine system. One reason for studying dopamine is that almost all drugs that are addictive cause problems with dopamine signalling. Another reason is that studying the very complicated pathways involved with dopamine has provided new therapeutic targets for patients suffering from dopamine abnormalities, such as Parkinson's disease.

Table 12.14 Cholinergic and adrenergic synapses

	Cholinergic	Adrenergic
Neurotransmitter	Acetylcholine (Ach)	Noradrenaline
System	Parasympathetic	Sympathetic
Effect on mood	Calming	Increased energy, alertness, and euphoria
Drugs increasing transmission at synapse	Nicotine	Cocaine and amphetamines

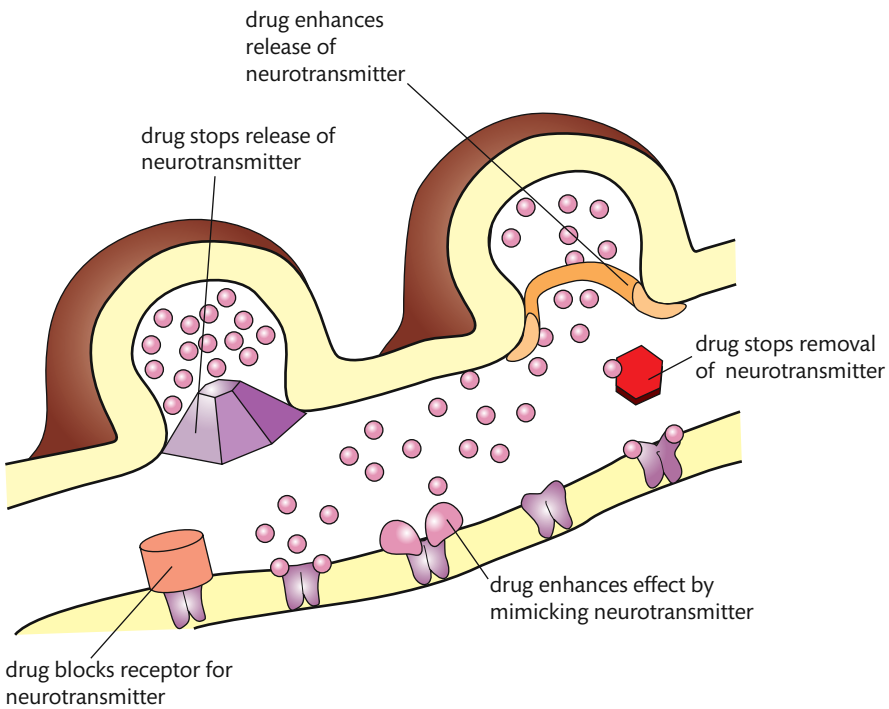


Figure 12.43 Drug action at the synapse.

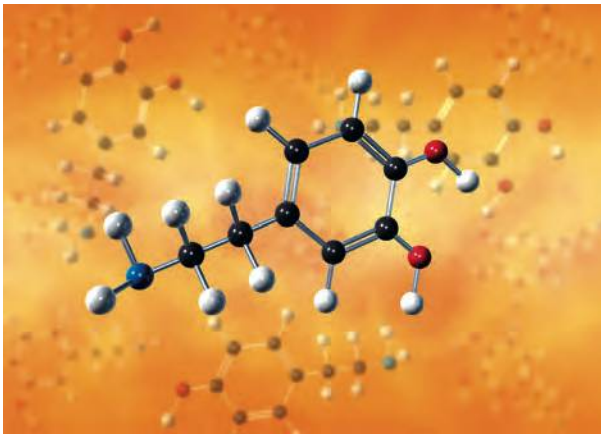
Researchers from Australia and Canada have found that alcohol and other drug abuse ranks among the top 10 contributors to the global burden of disease among adolescents. During the course of the study, alcohol contributed to 27% of the deaths of 15–29 year olds in economically developed countries.

Effect of drugs on the brain

Drugs can alter your mood or your emotional state. Excitatory drugs like nicotine, cocaine, and amphetamine increase nerve transmission, while inhibitory drugs such as benzodiazepines, alcohol, and tetrahydrocannabinol (THC) decrease the likelihood of

nerve transmission. Drugs act at the synapses of the brain using different mechanisms, resulting in an altered emotional state. Drugs can change synaptic transmission in the following ways (see Figure 12.43):

- block a receptor for a neurotransmitter (the drug has a structure similar to neurotransmitter)
- block release of a neurotransmitter from the presynaptic membrane
- enhance release of a neurotransmitter
- enhance neurotransmission by mimicking a neurotransmitter (when drugs have the same chemical structure as the neurotransmitter, they have the same effect but are not broken down as easily, so the effect is stronger because they stay longer in the synapse)
- block removal of a neurotransmitter from the synapse and prolong the effect of the neurotransmitter.



This is a model of dopamine. It is one of the most important of the neurotransmitters in the CNS. It plays a critical role in the way the brain controls movement, memory, and decision making. Excess dopamine may contribute to psychotic illnesses, notably schizophrenia. Underproduction of dopamine results in the movement disorder of Parkinson's disease.

Stimulants

Notice that the following drugs mimic the stimulation provided by the sympathetic nervous system, which we studied in Section 12.2. The sympathetic system is associated with 'fight or flight'. The sympathetic system is excitatory.

Nicotine in tobacco products is a stimulant that mimics acetylcholine. Thus it acts on the cholinergic synapses of the body and the brain to cause a calming effect. After acetylcholine is received by the receptors, it is broken down by acetylcholinesterase, but the enzyme cannot breakdown the nicotine molecules that bind to the same receptors. This excites the postsynaptic neurone and it begins to fire, releasing a molecule called dopamine. Dopamine gives you a feeling of pleasure. It is a molecule of the 'reward' pathway of your brain. Reward pathways are brain structures that induce pleasurable effects and when activated reinforce behaviour.

Cocaine stimulates transmission at adrenergic synapses and causes alertness and euphoria. It also causes dopamine release. Cocaine blocks removal of dopamine from the synapse so that it builds up. This leads to over stimulation of the postsynaptic neurone. Because this is in the reward pathway, it leads to euphoria. Notice that it acts the same way as nicotine. Both of these drugs can lead to addiction.

Amphetamine stimulates transmission at adrenergic synapses and gives increased energy and alertness. Amphetamine acts by passing directly into the nerve cells that carry dopamine and noradrenaline. It moves directly into the vesicles of the presynaptic neurone and causes their release into the synaptic cleft. Normally, these neurotransmitters would be broken down by enzymes in the synapse, but amphetamines interfere with the breakdown. Thus in the synapse high concentrations of dopamine cause euphoria, and high concentrations of noradrenaline may be responsible for the alertness and high energy effect of amphetamines.

Sedatives

Benzodiazepine reduces anxiety and can also be used to prevent epileptic seizures. Its effect is to modulate the activity of GABA, which is the main inhibitory neurotransmitter. When GABA binds to the postsynaptic membrane, it causes chloride ions (Cl^-) to enter the neurone. Remember that when Cl^- enters the neurone, the neurone becomes hyperpolarized and resists firing. Benzodiazepine increases the binding of GABA to the receptor and causes the postsynaptic neurone to become even more hyperpolarized (Figure 12.44).

Alcohol acts similarly to benzodiazepine in that it increases the binding of GABA to the postsynaptic membrane, and causes the neurone to become hyperpolarized. This explains the sedative effect of alcohol. It decreases the activity of glutamate, an excitatory neurotransmitter. Alcohol also helps to increase the release of dopamine by a process that is not well understood. It appears to stop the activity of the enzyme that breaks down dopamine in the synaptic cleft. Remember that dopamine works in the reward pathway.

Tetrahydrocannabinol (THC) is the main psychoactive chemical in marijuana. THC mimics the neurotransmitter anandamide. THC binds to the same receptor as anandamide (sometimes called cannabinoid receptors). THC is an inhibitory neurotransmitter and causes the postsynaptic neurone to be hyperpolarized. Scientists do not fully understand the role of anandamide, but it may play a role in memory functions. Marijuana disrupts short-term memory in humans. Anandamide may be involved in eliminating information from our memory that is not needed.

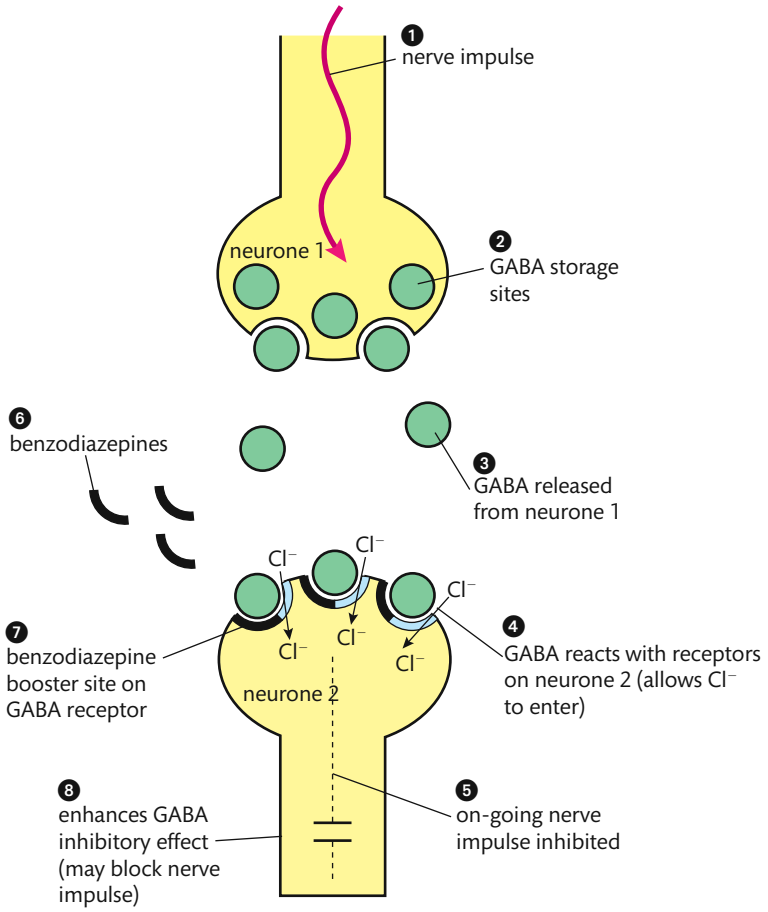


Figure 12.44 The effect of benzodiazepines at the synapse.

THC and cocaine affect mood, synapse, and behaviour

Marijuana users often describe the feelings produced by using the drug as being relaxing and mellow. Some say they feel lightheaded and hazy. The THC may dilate the pupils, causing colour perception to be more intense. Other senses may be enhanced. Some people experience a sense of panic and paranoia.

At the synapse, THC acts on cannabinoid receptors. These receptors affect several mental and physical activities, including:

- learning
- coordination
- problem solving
- short-term memory.

Because THC mimics anandamide, it inhibits the neurones that anandamide inhibits, but there probably isn't an enzyme for breaking down THC in the synapse. Its effect is much greater because it stays in the synapse longer.

High concentrations of cannabinoid receptors are found in the areas of the brain shown in Figure 12.45. The hippocampus is important for short-term memory. When THC binds to these receptors, it interferes with short-term memory. THC also affects coordination, which is controlled by the cerebellum and the basal ganglia. This is another reason for motor impairment when using marijuana.

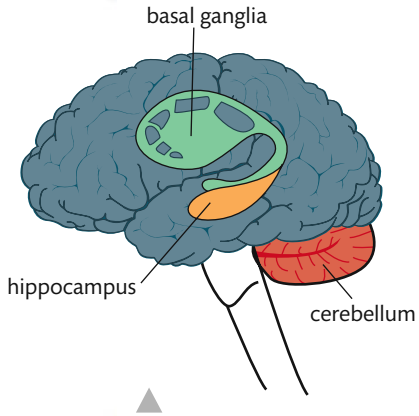


Figure 12.45 Areas of the brain with high concentrations of cannaboid receptors.

Some of the effects of cocaine are euphoria, talkativeness, and an increase in mental alertness. There is a temporary decrease in the need for food and sleep. Large amounts of cocaine can cause erratic and violent behaviour.

Cocaine affects the synapses that use dopamine as a neurotransmitter. Cocaine attaches to the dopamine receptors on the presynaptic membrane. It blocks the reuptake of dopamine so that it persists in the synaptic cleft. Cocaine is an excitatory neurotransmitter and causes constant stimulation of the postsynaptic neurone. Dopamine builds up in the synapse, causing feelings of euphoria.

Many drugs are therapeutic agents that rectify imbalances in chemical systems in the body. Here are two examples.

- Ritalin is used for the treatment of attention deficit hyperactivity disorder (ADHD). It increases dopamine levels by lessening its reuptake. This decreases the hyperactivity and allows patients to be more focused.
- Prozac inhibits reuptake of serotonin and is used to combat depression. Increased serotonin in the synapse results in improved mood.

Causes of addiction

Many drugs can lead to addiction: alcohol, tobacco, psychoactive drugs, and some pharmaceuticals. People take some drugs to alleviate symptoms of mental illness, and other drugs just for pleasure. The body often develops a tolerance and needs more and more of the drug to produce the same result. Addiction is a chemical dependency on drugs where the drug has 'rewired' the brain so that it has become an essential biochemical for the body.

Many people assume that smoking is just a bad habit. Scientific evidence shows that smoking causes the brain to be 'rewired'. Nicotine in tobacco products mimics

TOK

Make an argument for which addiction model is the most 'scientific.' Which model uses knowledge that has been collected scientifically according to the principles you have studied in your theory of knowledge course? Use the hotlinks at the end of this section to look at a range of models.

acetylcholine. It is not broken down easily and causes release of dopamine in the reward pathway. People who smoke are craving a dopamine spike.

Because the role of almost all commonly abused drugs is to stimulate the reward pathway located in the brain, withdrawal of the drug produces symptoms that are the opposite of euphoria. Common withdrawal symptoms are anxiety, depression, and cravings. With alcohol addiction, withdrawal symptoms include conditions that are sometimes fatal, such as seizures and delirium tremens (severe shaking). Continued addiction is even more harmful. Inhaled drugs can damage lungs. When sharing needles, addicts risk contracting human immunodeficiency virus (HIV) as well as hepatitis B and C. Kidney disease is also common.

Genetic predisposition

Evidence of a genetic predisposition to addiction has been found in studies of twins. Identical twins share the same genetic makeup, and fraternal twins have a 50% genetic similarity. Studies of male twins gives some support to the idea of a genetic predisposition, indicating that a genetically determined deficiency of dopamine receptors predisposes certain individuals to addiction. In one study, scientists compared genetically manipulated alcohol-preferring rats to normal rats. The alcohol-preferring rats had 20% lower levels of dopamine receptors than non-preferring rats. The alcohol-preferring rats consumed 5 g of ethanol per kilogram when given a choice between ethanol and water. The non-preferring rats consumed less than 1 g of ethanol per kilogram of body weight.

Social factors

Social factors can determine a child's vulnerability to substance abuse. Such factors include family, family parenting skills, and mental health problems of the family or child.

Behaviour is often tied to the peer group. Peer pressure is very influential on adolescents and, to a lesser extent, adults. Adolescents can be influenced or coerced into experimentation with drugs by their peer group. Users teach new users what effects to expect and what altered state is desirable. This social learning occurs with all types of drug use.

Rats are used in cocaine research to study addiction and other psychological effects of taking cocaine. Cocaine is an alkaloid drug that comes from the leaves of the coca plant, *Erythroxylon coca*. It produces feelings of exhilaration and energy. Prolonged cocaine use leads to psychological dependence. Overdoses can cause brain seizures or cardiac arrest.

When a drug is introduced into a culture, it can become a problem that did not previously exist. When the British introduced opium into China, it quickly became a major social problem. Heroin, introduced into the USA, has become a social catastrophe. The presence of alcohol at many social gatherings leads to the belief that alcohol must be available to have a good party. In Saudi Arabia, where the culture and the law prohibit alcohol use, alcoholism is rare.

Some research has shown that if drugs are cheap and easy to get a hold of, addiction is more likely.

Dopamine secretion

You will recall that dopamine is the neurotransmitter that activates the reward pathway and gives us a sense of pleasure or satisfaction. During cocaine use, dopamine builds up in the synapse.

With drug addiction, dopamine receptors are constantly stimulated. Overstimulation decreases the number of receptors, and the remaining receptors become less sensitive to dopamine. This process is called desensitization or tolerance. With tolerance, exposure to the drug causes less response than it previously caused. More and more of the drug is needed to have even a normal sense of well-being. This neuroadaptive change is probably critical for producing addiction.

Recently, scientists studying knockout mice (genetically manipulated mice addicted to cocaine) found another neurotransmitter that might be as important or more important than dopamine: glutamate (see Figure 12.46). Glutamate may 'oversee' the learning and memories that lead to cocaine-seeking. Further experiments will shed more light on this.

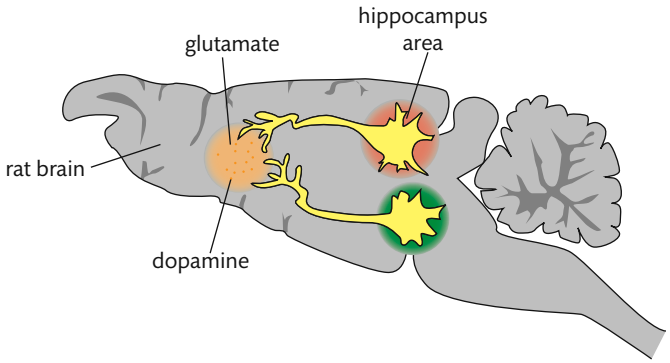


Figure 12.46 The role of glutamate in addiction: researchers found that stimulating neurones in the hippocampus (an area rich in glutamate) caused rats to search for cocaine.

While most modern governments have ruled hallucinogenic psychedelic drugs to be illegal, their use was not uncommon in ancient cultures. Psychedelic plants have been used in the past to induce a religious or mystical experience. Eastern Europe populations used mandrake, Asian populations used the opium poppy, and the Huicol in Mexico were fond of using peyote cactus. Some modern cultures still use drugs in religious and ritualistic ways. Here are two examples.

- Iboga is a root bark that is chewed by the Bwiti people of West Central Africa during rituals.
- Peyote is a cactus that is ingested during some Native American religious ceremonies. It is legal in the USA to use peyote if you are Native American and participating in religious ceremonies.

Anaesthetics

Anaesthetics are a very helpful type of drug that have been used by most people, even children. When you go to the dentist to have a tooth filled, you are glad that molecules have been discovered that can block the sensory reception of pain from travelling to the pain centres in the CNS.

If a message sent by a pain sensory receptor arrives at the CNS, you will feel the pain. Fortunately, we have Novocain. Novocain is a local anaesthetic that can block nerve transmission to the pain centres. Look back at the diagram of synaptic transmission (Figure 12.38). See where Na^+ diffuses into the neurone. When positive sodium ions are diffusing into the postsynaptic neurone, then the message is being carried along

the nerve. In this case, the pain impulse is travelling to the pain centre in the CNS. However, local anaesthetics like Novocain block the gated ion channel through which Na^+ is moving. No pain signals can be carried through to the CNS. Local anaesthetics produce drug-induced insensitivity to localized pain.

General anaesthetics are usually volatile compounds and inhaled, and thus affect the whole body. The result is a generalized insensitivity to pain. Normal heart rate and blood pressure are maintained but there is a reversible loss of consciousness. General anaesthetics are usually used in surgery; during the surgery the patient loses consciousness completely and has no awareness of pain.

Exactly how general anaesthetics work is not known, but recent studies indicate that they act on the CNS:

- by modifying the function of the gated ion channels in some fashion
- or by binding directly to the ion channels.

Both mechanisms prevent pain signals from travelling to the CNS.

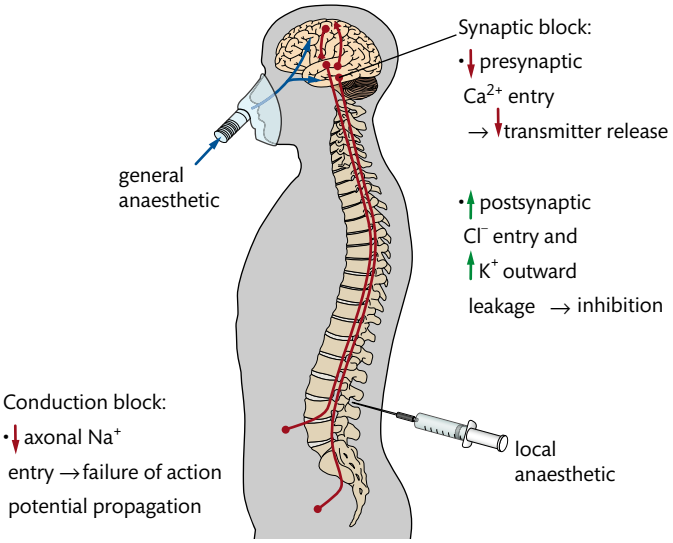


Figure 12.47 Anaesthetic suppression of the physiological response to surgery.

Figure 12.47 shows what is thought to be the mode of action of a general anaesthetic. The general anaesthetic changes the nerve cells so that normal communication among many of them is closed off for a period of time. Anaesthetics can achieve this is by altering the ion channel. Sensations of all kinds are temporarily blocked from reaching the brain.

Endorphins

Endorphins were first discovered by scientists studying opium addiction. They found receptors for opiates, morphine, and heroin in brain cells. It may seem odd that brain cells have receptors for molecules made by plants (the opium poppy). Scientists found that morphine and heroin bound to the brain receptors because they were mimicking endorphins. We now know that endorphins are CNS neurotransmitters with pain-relieving properties.

- They are released by the pituitary gland during stress, injury, or exercise.
- They are small peptides that bind to opiate receptors.

- They block transmission of impulses involved in pain perception.
- They bind to the receptors in the membrane of neurones involved in pain perception, and block the release of neurotransmitters.

MDMA

MDMA and serotonin

You may be asked to evaluate data showing the impact of MDMA (ecstasy) on serotonin and dopamine metabolism in the brain. In order to evaluate such data, a basic understanding of the action of MDMA on serotonin and dopamine metabolism in the brain is needed.

MDMA is structurally related to amphetamine. Both are drugs that affect transmission at the synapse. MDMA is a synthetic molecule that causes extra serotonin to be released, giving the user a pleasurable feeling. The cell bodies of serotonin neurones are in the brainstem and have a network of axons throughout the brain.

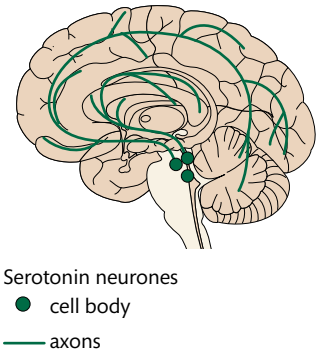


Figure 12.48 Serotonin neurones in the brain.

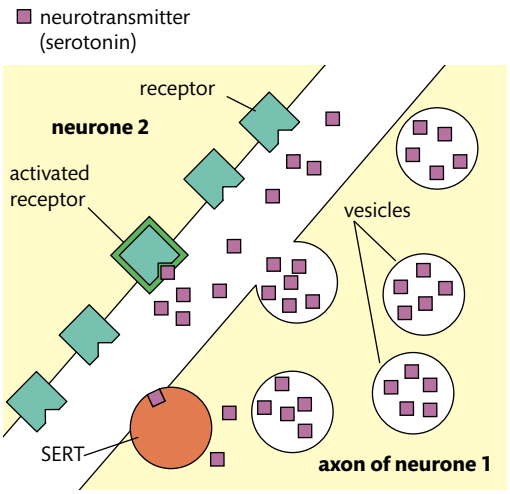


Figure 12.49 Serotonin in the synapse.

MDMA initially affects the serotonin neurones in the following manner.

- The axon of neurone 1 releases serotonin into the synapse.
- The receptor on neurone 2 is activated by the serotonin.
- A SERT (serotonin transporter) vesicle on neurone 1 will take up all the extra serotonin in the synapse and pump it back in when needed.

- MDMA forces serotonin axons to release lots of serotonin. This gives you a great feeling! Pretty soon SERT catches up. The good feeling is then replaced by depression.
- People who take MDMA (ecstasy) all night long will eventually deplete their serotonin levels.

The term 'endorphin' means the morphine within. The pain-relieving properties of endorphins are similar to those of morphine.

MDMA and dopamine

The theory of action of dopamine is as follows.

- When serotonin is depleted, the SERT receptors (vacuums) are empty.
- Dopamine enters the SERT receptors by mistake.
- Dopamine is broken down and the products are toxic to the serotonin-producing neurones.
- Neurotoxicity can cause long-lasting damage to brain cells, killing them or impairing their function.

CHALLENGE YOURSELF

Look at the Figure 12.50. It shows the number of receptors for serotonin that are active over time in a group of rats that were previously given eight doses of MDMA over a period of 4 days. Notice that a control group received no MDMA. The molecule named [1215]MIL was used to label active serotonin receptors in the brains of the rats.

Answer the following questions from the data shown in the graph.

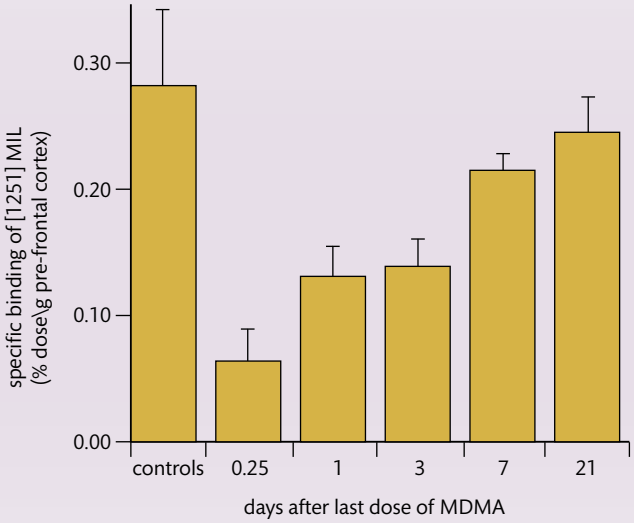


Figure 12.50 The effect of MDMA on the number of available serotonin receptors in the brains of a group of rats. MIL is a molecule used to label active serotonin receptors. Scheffel et al. 1992

Write the answers to questions as fully as you can. Facts score points in a biology exam. But be specific, not general, when writing your answers.

17 Looking at the labels on the graph, what does 'specific binding of' tell you about the number of active receptors?

18 Which group has the highest number of activated serotonin receptors? Give evidence.

19 Compare the data for 0.25 days after the first dose with the data for 1 day after the first dose.

20 What happened to the receptor density over the 21 days following the last dose of MDMA?

21 Explain how MDMA acts on the receptor density of these rats 8 hours after the last dose of MDMA.

CHALLENGE YOURSELF

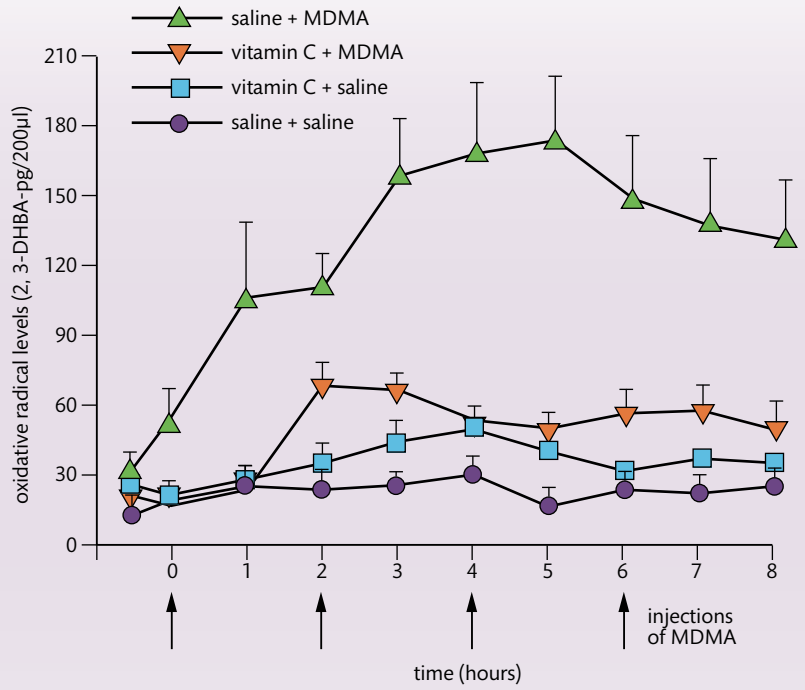


Figure 12.51 Effect of vitamin C and MDMA on free radical levels in rats. Shankaran et al. 2001

Some experiments have documented that molecules called antioxidants can reduce MDMA-caused neurotoxicity. Interesting work has been done with cheap antioxidants such as ascorbic acid (vitamin C). High levels of oxidative radical levels are known to cause neurotoxicity. Look at Figure 12.51 and answer the following questions.

22 Which two groups are the 'control' rats? Explain why.

23 Compare the other two groups and explain the findings.

24 Draw a conclusion based on these data.

NATURE OF SCIENCE

There has been controversy about the methods of diagnosis and treatment of attention deficit hyperactivity disorder (ADHD) since the 1970s. Treatment includes the use of stimulant medications. The majority of people diagnosed with ADHD are children. Because of their age, whether or not they are given the drugs is decided by their adult legal guardians. The stimulant drugs given have some undesirable side-effects and uncertain long-term effects. Ethical issues also arise when groups or individuals who are receiving money from pharmaceutical companies actively promote these drugs.

Is there enough scientific data to back up the diagnosis of ADHD? Some prominent scientists say there is not. Brain-imaging studies have been carried out that indicate there may be slight differences in the brain of a person with ADHD compared with a person without symptoms of ADHD, but the diagnosis is usually based on behaviour alone. Is that enough? What scientific data are used to justify a diagnosis and give a child a drug that is possibly harmful?

To learn more about the action of drugs, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.5.

Exercises

19 Outline summation as a function of postsynaptic neurones.

20 Describe the two methods by which slow-acting neurotransmitters modulate fast synaptic transmission in the brain.

21 Explain how memory and learning are affected by slow-acting neurotransmitters.

22 Outline the effect of anaesthetics on awareness.

NATURE OF SCIENCE

Testing a hypothesis: experiments to test hypotheses on the migratory behaviour of blackcaps have been carried out.

A.6 Ethology

Understandings:

- Ethology is the study of animal behaviour in natural conditions.
- Natural selection can change the frequency of observed animal behaviour.
- Behaviour that increases the chances of survival and reproduction will become more prevalent in a population.
- Learned behaviour can spread through a population or be lost from it more rapidly than innate behaviour.

Applications and skills:

- Application: Migratory behaviour in blackcaps as an example of the genetic basis of behaviour and its change by natural selection.
- Application: Blood sharing in vampire bats as an example of the development of altruistic behaviour by natural selection.
- Application: Foraging behaviour of shore crabs as an example of increasing chances of survival by optimal prey choice.
- Application: Breeding strategies in coho salmon populations as an example of behaviour affecting chances of survival and reproduction.
- Application: Courtship in birds of paradise as an example of mate selection.
- Application: Synchronized oestrus in female lions in a pride as an example of innate behaviour that increases the chances of survival and reproduction.
- Application: Feeding on cream from milk bottles by blue tits as an example of the development and loss of learned behaviour.

Guidance

- *The seven applications in this sub-topic are intended to reinforce understanding of the general principles. The applications include a range of types of behaviour and types of animal. Other examples, including local examples that can be observed, should also be studied if possible.*

Some ethologists have dismissed psychology as irrelevant because it is based on animal behaviour in an unnatural setting. Psychologists have accused ethologists of ignoring the concepts of learning and motivation and placing too much emphasis on instinct. Explore the different ways scientific method is used in each of these disciplines. In your opinion, is one more 'scientific' than the other?

TOK

Ethology versus psychology

Ethology is the study of animal behaviour in natural conditions. Consequently, ethologists typically work 'in the field', observing behaviour in such a way that their subjects are affected by their activities as little as possible. Psychologists adopt an experimental, laboratory, approach in which the external world of the animal is controlled carefully. Remember the experiment in conditioning carried out by Pavlov and the dogs he conditioned to salivate in response to a bell. Pavlov was a psychologist. Jane Goodall, who is famous for observing the behaviours of chimpanzees in their natural environment, is an ethologist. The difference between the two approaches to studying animal behaviour is summarized in the following sentence. 'A psychologist put the animals in a box and watches them from the outside; an ethologist put himself or herself in a box and looks out at the animals.'

Learned versus innate behaviour

As we have discovered in last few sections, learned behaviour is not genetically programmed. Learned behaviour is the process of gaining knowledge or skills. A skill can be improved with practice. Knowledge is measured by performance. Learned behaviour is dependent on the environmental context. If the context is missing, the skill may disappear over time. Innate behaviour is genetically programmed. It is as ingrained in an animal as much as the colour of its feathers or hair. Innate behaviour is encoded in DNA and can only be changed by genetic change. This type of change would take place over many generations.

Natural selection

Animal behaviour is more than just single reflexes. It is a complicated series of responses to the environment in which animals live. Exactly which animals survive and which do not is determined by their surroundings and the compatibility of their characteristics with those surroundings. Scientists studying animal behaviour have observed that some populations of animals have changed the frequency of their behaviour. For example, some birds may migrate earlier, some salmon may mature quicker, some birds may develop more extreme courtship patterns. These behavioural changes may be so extreme that a new species is formed, as might eventually occur with European blackcaps. Through careful observations, ethologists have collected data that show that natural selection, which increases the chances of survival and reproduction, has acted on genetically caused behaviours. This has made those genetically caused behaviours more prevalent in a population.

Fitness in this context means the ability to survive and reproduce. It means the relative contribution that the organism will make to the gene pool of the next generation.

TOK

There are many poor examples of supposed links between animal responses and natural selection. It is easy for us to guess how the behaviour of an animal might influence its chance of survival and reproduction, but experimental evidence from carefully controlled trials is needed to back up our intuition. Discuss the difference between evidence that shows correlation and evidence that shows causation. What correlation evidence was observed in the example of UK blackcaps? How was evidence of causation collected?

Migration in European blackcaps

Figure 12.52 This map shows the two different blackcap migration routes. http://evolution.berkeley.edu/evolibrary/news/060101_batsars

As we realize the importance of bird migration to bird survival and reproduction, it is exciting to see that international organizations are recognizing the need to conserve migration routes. In November 2006, the United Nations (UN) launched its project on bird migration routes in Africa and Eurasia. The name of the project is Wings Over Wetlands (WOW). This project aims to conserve critical areas needed by waterbirds migrating across these continents.

TOK

What will happen when global warming causes large migrations of fish and other animals in the ocean? Will this migration also cause new species to emerge? The impact that global warming is beginning to have on Australian marine life has been announced by the Great Barrier Reef National Park. Fish and seabirds are being driven south. Coral reefs are threatened. Warmer oceans can affect reproductive cycles. Nesting turtles are particularly vulnerable.

Birdfeeders may have split the blackcap into two species.

European blackcap birds are named for the distinctive black cap of the male which contrasts with the otherwise greyish plumage.

Let's consider the interesting case of a bird called the European blackcap, *Sylvia atricapilla*. These birds are small warblers that usually migrate between Spain and Germany. They breed in Germany in the spring and summer, and spend the winter in Spain. About 50 years ago, ornithologists (bird watchers) noticed that some blackcaps were going to the UK for the winter instead of Spain. Ornithologists began studying blackcap behaviour. The ornithologists noticed that the UK blackcaps left to go back to Germany 10 days earlier than the Spanish blackcaps. They also noticed that the earlier the birds arrived in Germany, the more choice of territory they had, and the more eggs they laid. The UK blackcaps had a distinct advantage over the Spanish blackcaps.

In order to determine whether this behaviour had a genetic basis, scientist Peter Berthold carried out a series of experiments. Some eggs were collected from parent birds who had been in the UK the previous winter, and some eggs were collected from parent birds who had been in Spain the previous winter . The young were reared by the scientist and the direction of their subsequent migration recorded. No parents were around to teach the young birds what direction to fly. All of the birds in the study tended to migrate in the same direction as their parents had done. This supports the hypothesis that blackcaps are genetically programmed to fly in a certain direction.

In another experiment, Berthold interbred birds from both groups. Can you guess what happened? The offspring followed a route halfway between each of the parents. This supported the hypothesis with even more data that the travel direction is genetically set.

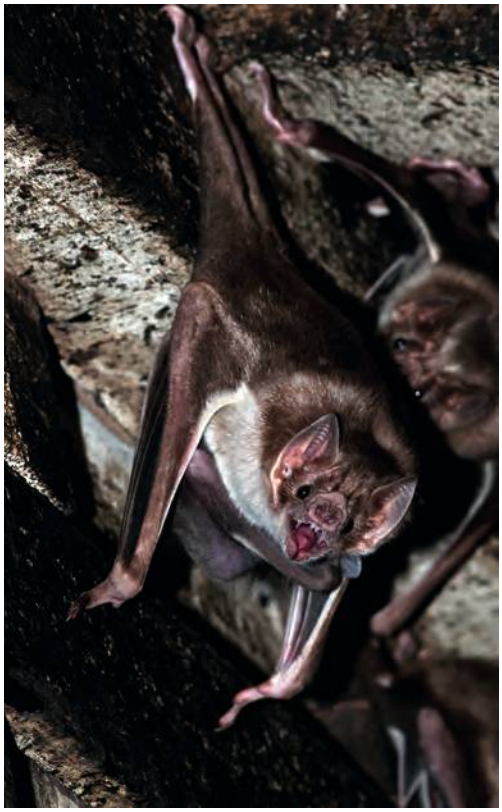
What was the original reason for some birds to go to the UK? Could it have been a mutation that caused a change in the travel direction? Usually mutations are not beneficial to an organism that is well adapted. In this case, however, humans may have played a role in the outcome. When some of the birds arrived in Britain instead of Spain, they found many UK gardens with winter bird feeders available to them. The behaviour of UK bird lovers may have been the environmental factor that has made UK blackcaps so successful, when often going to the wrong place can be deadly.

Another scientist, Gregor Rolshauer, from the University of Freiberg, has shown that the blackcaps are now two populations separated by the timing of their breeding. UK blackcaps get to Germany 10 days earlier than Spanish blackcaps, and are already

breeding before the Spanish blackcaps arrive. The two populations are even becoming physically different. For example, the UK blackcaps have a sharper, longer beak, perfect for eating seeds from a winter garden feeder. The Spanish blackcaps have a shorter, stronger beak for eating the fruit that is their natural diet in Spain. These differences have arisen in just 30 generations. This is an excellent example of the genetic basis of behaviour and its change by natural selection.

Blood sharing in vampire bats

In the evening, vampire bats fly across the landscape and search for warm-blooded prey. After several hours the bats return to their roost, feed their young, and interact with nest mates. Some recent studies have shown that female bats regurgitate blood to long-term associates on a regular basis. The regurgitation of blood to one another significantly increases their chances of survival.



Female vampire bats feed their young with regurgitated blood.

Why would helping a fellow individual survive be beneficial to your own survival? A study on vampire bats was carried out at the University of Florida to answer that question. It showed that a vampire bat will die if it does not have food for two nights in a row. Because it can be difficult to find a meal, 30% of bats in a cluster (group of bats) fail to find food in any given evening. Fortunately for them, they receive a meal regurgitated from an altruistic bat.

Food sharing appears to be altruistic. An altruistic behaviour is one that benefits others at a cost to oneself. A donor bat gives food to a recipient bat whose chances for survival are then increased. This behaviour does not seem to increase the survival of the donor bat directly. Often acts of altruism are actually a case of kin selection, in other words helping relatives, which actually helps the donor's own genes survive in a population. However, there is evidence that many of the nest mates of bats, for example, are not relatives. This is then called reciprocal altruism. When

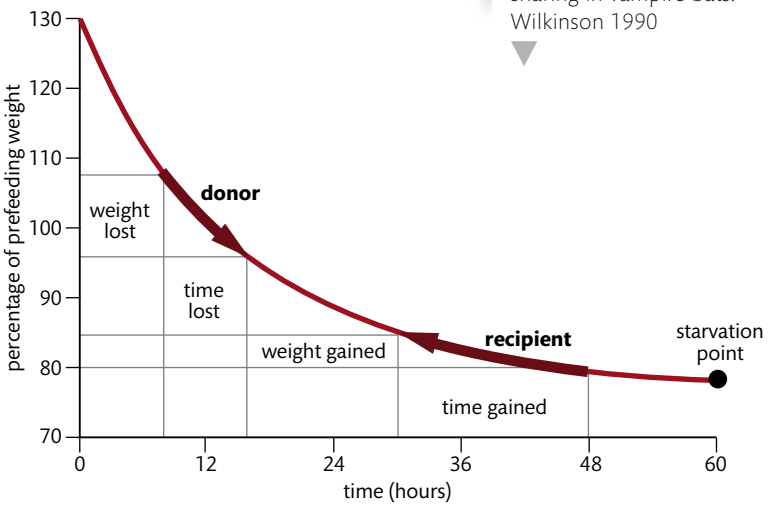


Figure 12.53 Blood sharing in vampire bats. Wilkinson 1990

individuals who are on an equal basis share resources with each other, it becomes an advantage for both. One study has shown that bats are more likely to share food with others that have recently shared food with them. Regurgitation of blood to close fellow bats seems to be an altruistic behaviour that has developed by natural selection. The selection factor is that those who share blood with close associates are those that survive to reproduce. Thus the genes controlling this behaviour continue to be inherited within the population.

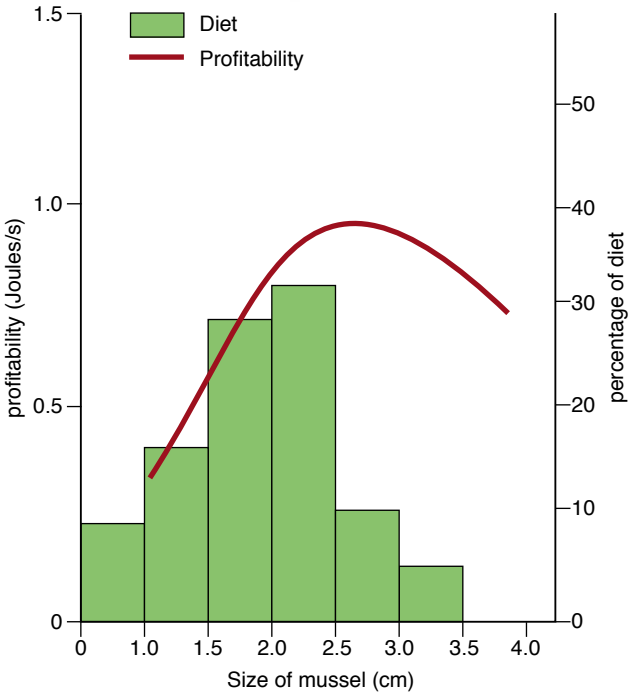


Figure 12.54 Optimal foraging in shore crabs. http://biology-forums.com/index.php?action=gallery;sa=view;id=1361

Foraging behaviour in shore crabs

Observations of shore crabs, which prey on mussels, have shown that crabs choose a mid-size mussel to prey upon rather than a larger mussel. Why does it make this prey choice? When a crab selects a mussel as prey, it must weigh up the cost compared with the benefits. You might think that the yield in food energy would be the top priority. However, scientists studying shore crabs now have a new hypothesis. Evidence is being collected that indicates that, although larger mussels are available and could be crushed and eaten, thereby providing more food, selecting small, more easily crushable, mussels prevents wear and tear on a crab's claws.

During their mating season, shore crabs congregate in certain areas. Here male crabs fight furiously over access to females. Undamaged and large males are much more likely to mate with available females than others. The risk of claw damage is therefore of greater importance in terms of reproductive success than the short-term energy gain of eating a mussel.

Breeding strategies in coho salmon

A number of strategies can exist for passing on genes. The strategies used by the coho salmon involve both the bright coloured, fierce and large 'hooknose' males and the smaller, camouflaged, sneakier 'jack' males. Which strategy works the best? Behavioural ecologists originally assumed that the large, muscular males (the hooknoses) were superior. Further studies have shown this may not be the case: the behaviour strategy of the jacks may be more successful. Jacks are smaller as juveniles and develop in a shallow area of the stream called a riffle. The riffle contains abundant food. This allows the jacks to grow quickly and head out to sea earlier. They return to spawn with females at least one year earlier than the hooknoses. Over many generations, the females can produce more offspring. The females can pass on the traits that lead to a shorter maturation time in the males.

A brightly coloured hooknose salmon swimming upstream.

It seems that the quiet strategy pays off. Observations have revealed that jacks will sneak up on a female while the larger hooknoses are fighting with each other. Jacks are not brightly coloured like the hooknoses, and are not so easily seen. Jacks mating with females are also less aggressive. This seems to increase spawning time and causes the females to lay more eggs. In one observation, 75% of jacks mated with females compared with only 58% of hooknoses. Both strategies work, but having two strategies increases the genetic variation in a population.

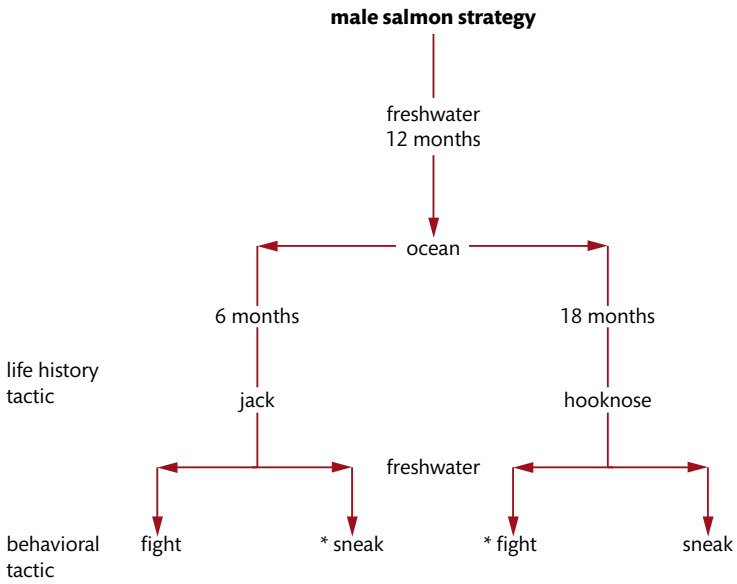


Figure 12.55 The two breeding strategies in male coho salmon.
Gross 1991

Courtship in the bird of paradise

Mate selection is important for survival and reproduction in a species. Many species of birds of paradise have evolved on the island of New Guinea, a place with abundant food and no mammalian predators. This group of birds is one of the most prominent avian examples of sexual selection.

In order to appreciate the extreme mating behaviour that a male bird of paradise undertakes, use the hotlinks at the end of this section and watch any video on courtship behaviour. You will be amazed!

Female birds of paradise are much less coloured and feathered than the males. The males display extreme colour, plumage, and fancy behaviour to show off in front of the females. The condition of his plumage and quality of his colour tells a female whether the male is healthy and will produce healthy offspring. Most males are extravagantly beautiful and display behavioural oddities such as puffing up their bodies, turning feathers into skirts, and dancing in dizzying circles.

A bird of paradise.

Nearly all birds of paradise court potential mates with a movement and courtship display. There are almost 40 species of bird of paradise, and all have different displays and colourings. A male *Carola parotia* does a dance where his left flank feathers form a skirt. A male blue bird of paradise, *Paradisaea rudolphi*, hangs upside down on a branch and spreads out his blue feathers. If a particular dance becomes common place in a species, it is because that specific courtship display has evolved over time based on the positive response of the females of that species.

All of these attractive features are genetically determined. The traits are passed down to male offspring and become even more prominent in the species over generations. This is sexual selection. Females are looking for males with the most vigour and the most fitness: the courtship dance proves this vigour and fitness to the female. Because it is the females who decide who to mate with, the female behaviour dictates how the males evolve.

Oestrus in female lions.

A blue tit pecking the silver foil top of a milk bottle to reach the milk underneath had become part of British folklore, but it is a sight that is rarely seen nowadays. So how did this phenomenon occur and why is it something you no longer see?

Synchronized oestrus in lions

In a study of 15 lion prides in Serengeti National Park, it was shown that when a pride of female lions is taken over by new males, the reproductive state of the females is synchronized. When the new males move in, they kill or chase out all the cubs. Loss of their cubs causes all the females to move into oestrus within 2 weeks. This is an innate, synchronized response to the loss of the cubs. Oestrus is a period of sexual receptiveness. When a female is in oestrus, she gives off a scent. The males then follow her around until she is ready to mate.

But what is the benefit to the pride (females) of being in oestrus at the same time? Can it help the females' genes pass on to the next generation? All the females in a pride are usually related, as aunts, nieces, or sisters. Female lions are the main hunters and hunt in groups. They take care of the cubs in groups; all lionesses suckle all cubs and protect all cubs. All the cubs are raised in a community; if one lioness dies, another female can raise the cub. In other words, all the females in the pride are protecting the genes they share in common.

Life as a cub is dangerous. Death from infanticide because of pride take over is common, and cubs are vulnerable to predators such as hyenas. Is it beneficial for all the cubs in a pride to be at almost the same age as a result of the synchronization of the female oestrus? If the cubs in a pride are all at different ages, the variety in size can cause younger ones to be bullied and harmed by older cubs. It is more difficult for a female to hunt and patrol if the cubs are at different ages and some are too young to be left alone. Studies have shown that when birthing of the cubs occurs at the same time, it has a distinct advantage because it increases the survival rate of the cubs. If the cubs survive to sexual maturity, the genes they carry then have a higher chance of being inherited in subsequent populations of lions. This innate behaviour of synchronized oestrus cycle increases the chances of survival and reproduction of offspring, carrying the genes of both the males and the females involved into the next generation.

Blue tits and learning

Can animals learn a skill and then forget it? It would appear so. The story of the blue tits in Britain provides some clues about this process of learning and forgetting. At the beginning of the 20th century, milk was commonly delivered to the doorsteps of British residents in bottles, and the milk had a layer of cream that settled on the top. (At that time the mechanism, homogenation, of mixing the cream and milk so that they do not separate was not common.) Blue tits would

suck the cream from the tops of the bottles before the bottles were taken inside. Another bird that did this was the European robin.

When the dairy industry started to cover the bottles with an aluminium sealed top, only the blue tits learned to remove the bottle tops to get to the cream; the robins did not. A few robins could remove the tops, but it did not become a common behaviour in robin populations. However, it did become an almost universal behaviour within blue tit populations across Britain. Scientists became interested in how this learned behaviour had spread.

Data were collected that demonstrated that blue tits normally peel bark off trees to look for insects. This is similar to the technique they used to peel the top off a milk bottle. What about the robins? It seems that robins are solitary birds, while blue tits are flock birds. Can part of the answer be that the learning was propagated socially in the flock? Researchers in Canada and Austria set up experiments to test the learning of blue tits and to try and determine whether the learning was social. All the experiments supported the view that the bottle opening was a combination of innovation by some blue tits and learning spread by social communication.

Recently, more studies have been carried out that indicate that this learning, which was once so beneficial to the blue tits, has now been lost. What changed? It seems that the behaviour has been suppressed because of changes in the:

- style of milk (whole milk is now homogenized so that the cream does not separate and rise to the top)
- style of the container (it is difficult for the birds to remove the plastic tops)
- delivery method (most people now buy their milk at a shop rather than have it delivered).

This seems to be an excellent example of how some birds developed a learned a behaviour, then, when the conditions were altered, the behaviour was lost.

If blue tits get past the cream to the milk, they are lactose intolerant and get bad diarrhoea from drinking the milk.

CHALLENGE YOURSELF

Using the topics covered in Section A.6, summarize the example that fits the following principles best.

25 Innate behaviour increases the chances of survival and reproduction of offspring.

26 Learned behaviour can spread through a population or be lost from it more rapidly than innate behaviour.

27 The genetic basis of behaviour can change due to natural selection.

28 Optimal prey choice can increase chances of survival and thus the ability to pass on your genes.

29 Breeding strategies can affect chances of survival and reproduction.

30 Mate selection can be determined by courtship behaviour.

31 The development of altruistic behaviour as acted upon by natural selection can benefit all individuals involved in the long term.

To learn more about birds of paradise and their courtship behaviour, go to the hotlinks site, search for the title or ISBN, and click on Chapter 12: Section A.6.

Exercises

23 Describe how natural selection can change the frequency of observed animal behaviour.

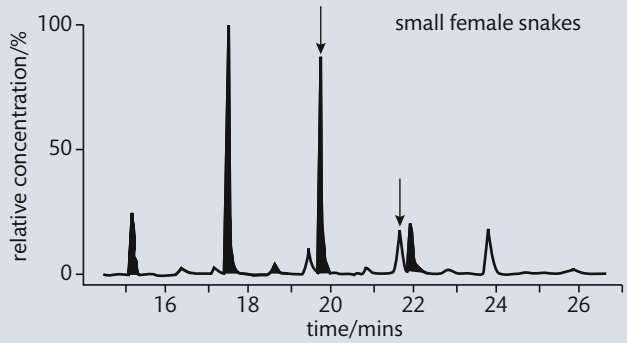
24 Outline an example of behaviour that will become more prevalent in a population since it increases the chances of survival and reproduction.

Always define your terms at the beginning of your answer.

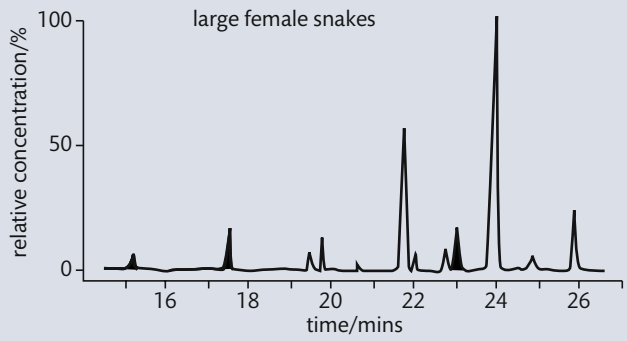
Make sure you are answering the whole question, not just telling a story. For example, question 27 in Challenge yourself is asking about genetic behaviour and natural selection.

Practice questions

1 In many vertebrate species, individuals of one or both sexes select for some features among potential mates in an effort to optimize their reproductive success. Sex pheromones are chemicals that help in chemical communication between individuals of the same species. The male red-garter snake (*Thamnophis sirtalis*) displays a courtship preference for larger female snakes. Researchers tested the hypothesis that males could distinguish among females of varying size by the composition of the skin lipids that act as pheromones.
Skin lipid samples were collected from small females (46.2 ± 2.7 cm in length) and large females (63 ± 2.6 cm in length). The samples were analysed by gas chromatography and the relative concentrations of saturated and unsaturated lipids were determined. The graphs show the time profiles when different lipids emerged from the gas chromatography column.
The shaded peaks represent saturated lipids and the unshaded peaks represent unsaturated lipids.



LeMaster and Mason 2002



LeMaster and Mason 2002

(a) Using the graph for large female snakes, state the relative concentration of the unsaturated lipid corresponding to the peak at 26 minutes. (1)

(b) Using the graph for small female snakes, calculate the ratio of unsaturated to saturated lipids indicated by the arrows. (1)

(c) Compare the pheromone profile of large female snakes with the profile of small female snakes. (2)

(d) (i) Suggest an experiment to test the hypothesis that the male red-garter snake could discriminate between larger and smaller female snakes. (2)

(ii) Suggest an advantage for male snakes selecting larger females. (1)

(*Total 7 marks*)

2 **(a)** Define the term innate behaviour. (1)

(b) Discuss the use of the pupil reflex for indication of brain death. (2)

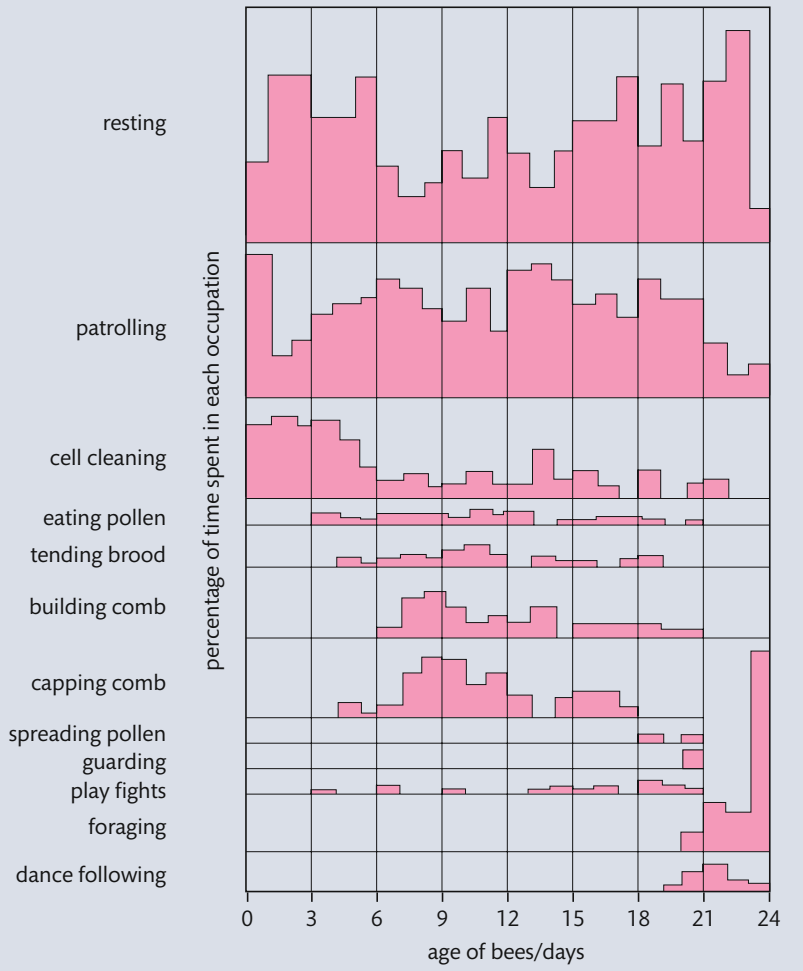
(*Total 3 marks*)

3 **(a)** Explain the effects of excitatory psychoactive drugs using two named examples. (6)

(b) Compare the roles of the parasympathetic and sympathetic nervous systems. (4)

(*Total 10 marks*)

4 During the first 24 days, worker bees (*Apis millifera*) go through a series of occupational specializations. The diagram below is a record of the first 24 days in the life of one worker bee. Adding the heights of the bars for a particular day gives 100% of the activity for that day.



Gould 1992

(a) (i) Determine the percentage of time the bee spent on cleaning on day 1. (1)

(ii) Calculate the ratio of time spent foraging to the time spent patrolling on day 24. (1)

(b) Identify the two most common activities of the bee over the 24 days. (1)

(c) Other than resting and patrolling, describe the changes in the bee's activities over the 24 days. (3)

(d) Suggest why patrolling is a social behaviour. (1)

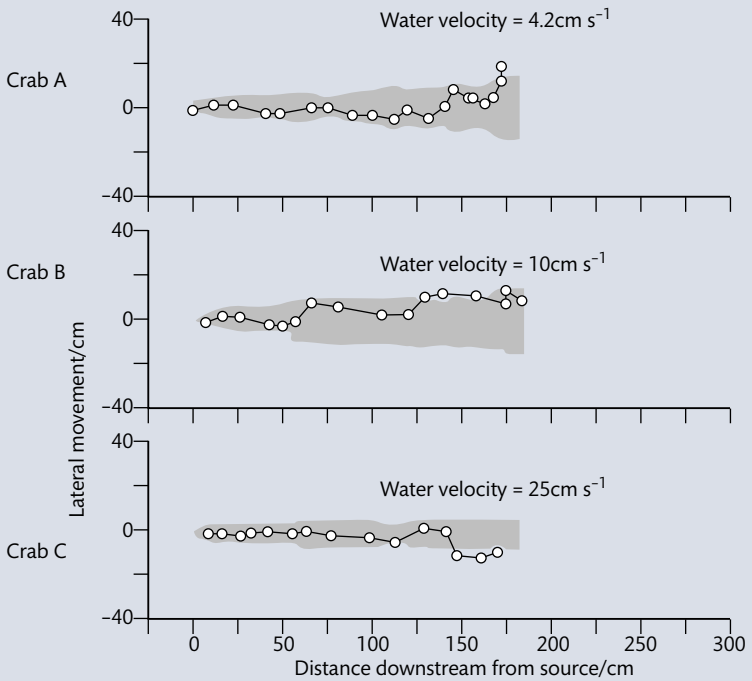
(*Total 7 marks*)

5 Outline Pavlov's experiments on the conditioning of dogs.

(*Total 3 marks*)

6 Blue crabs (*Callinectus sapidus*) hunt clams in river streams. In response to being attacked the clams release chemicals. The hunting behaviour of the blue crabs was studied by recording their movements after the release of the chemical, which was visualized by adding a dye (noted by shading in the figure below). The behaviour was studied and recorded under three different water velocities (expressed in cm s^{-1}).

Each graph below shows the movement of a single crab recorded at 1 s intervals, as it moves upstream towards the source of the chemical.



Adapted from Zimmer-Faust et al. 1995

(a) Identify which crab shows the greatest lateral movement. (1)

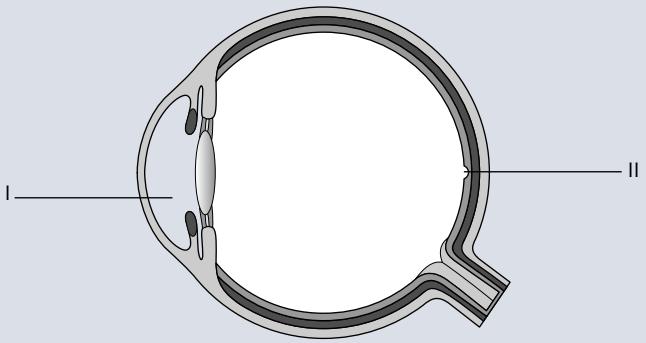
(b) Calculate the greatest speed of crab movement at 150 cm from the source of the chemical. (1)

(c) Compare the effect of water velocity on the hunting behaviour of blue crabs. (2)

(d) Discuss two other factors that could influence the outcome of this experiment. (2)

(*Total 6 marks*)

7 **(a)** Label the diagram of the human eye shown below. (2)

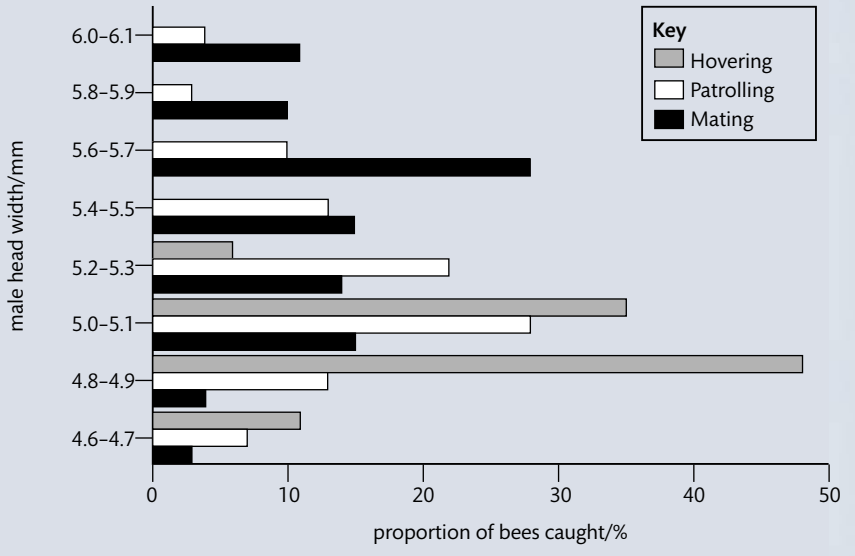


Jones and Jones 1997

(b) State **one** effect of the parasympathetic nervous system. (1)

(*Total 3 marks*)

8 In the bee *Centris pallida*, the male performs one of two mating behaviours known as patrolling or hovering. Patrolling bees search close to the ground, waiting to mate with virgin females as soon as they mature and emerge from their burrows. When a female emerges, the patrolling males spend so much time fighting among themselves that often none of them mate with the female so it flies to a tree to feed. The hovering bees fly higher than the patrolling bees, or fly around the trees. When a hovering bee sees a female he pursues her and tries to mate with her. Scientists caught 100 hovering bees, 250 patrolling bees and 150 mating bees and measured the width of the head of each bee.



Chapman and Reiss 1999

(a) Identify the largest head width range found in the sample of hovering bees. (1)

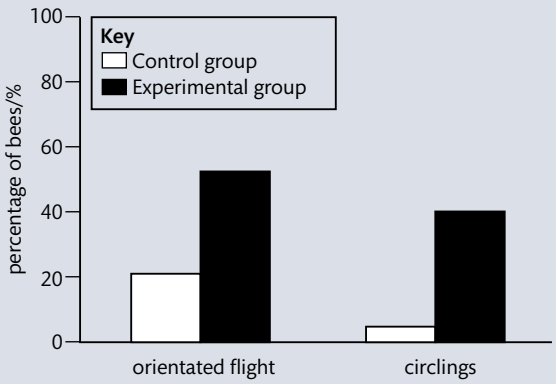
(b) Calculate the number of mating bees with head width from 5.8 to 5.9 mm. (1)

(c) Deduce the relationship between head width and mating success. (2)

(d) Suggest why bees with small heads tend to hover rather than patrol. (1)

(*Total 5 marks*)

9 Evidence suggests that the behaviour of bees is often a response to odours. Scientists placed bees 200 cm away from an attractive odour source. An experimental group of bees had previous exposure to the odour, a control group had no previous exposure. Both the percentage of bees flying towards (orientated flight) and the percentage circling the odour source were measured.



Adapted from Chaffiol et al. 2005

(a) Calculate the percentage increase in orientated flight between the control group and the experimental group. (1)

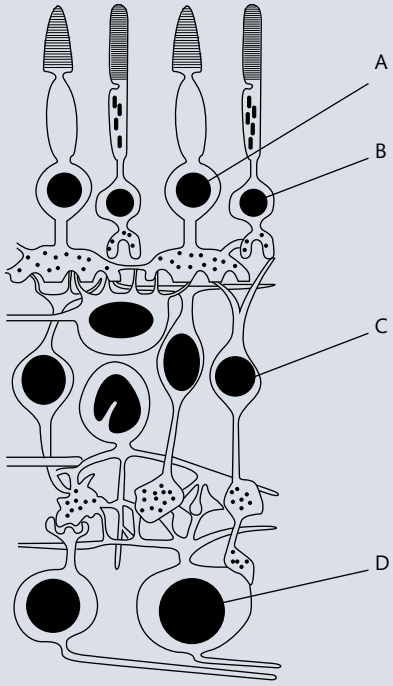
(b) Describe the effect of previous exposure to the odour on the flight of bees. (2)

(c) Outline the type of behaviour that the experimental group demonstrates. (1)

(d) Discuss the implications of this study for the survival of bees. (3)

(*Total 7 marks*)

10 (a) Label the diagram of the retina below. (2)



Adapted from Dowling and Boycott 1966

(b) Draw an arrow on the diagram above to indicate the direction in which light is moving. (1)

(c) Compare the functions of rod and cone cells. (3)

(Total 6 marks)

13 Option B: Biotechnology and bioinformatics

Nucleotides of the human genome.

Essential ideas

B.1 Microorganisms can be used and modified to perform industrial processes.

B.2 Crops can be modified to increase yields and to obtain novel products.

B.3 Biotechnology can be used in the prevention and mitigation of contamination from industrial, agricultural, and municipal wastes.

B.4 Biotechnology can be used in the diagnosis and treatment of disease.

B.5 Bioinformatics is the use of computers to analyse sequence data in biological research.

Biotechnology has been used for centuries to bake bread, make cheese, and brew alcoholic beverages. However, recent developments in biotechnology have given the term a new meaning. Modern biotechnology has captured the attention of everyone. Modern biotechnology offers us the chance to make dramatic improvements in industry, agriculture, medicine, and environmental science. Bioinformatics is the workhorse of biotechnology, and includes processes such as data mining and managing databases of biological information.

Because microorganisms are so metabolically diverse and have a fast growth rate, they can be invaluable to us. With genetic engineering we have accomplished mass production of penicillin, a key antibiotic; mass production of citric acid, one of the most widely used food-flavouring agents; and the production of biogas, which could be a main energy source of the future.

You may have heard of GMOs (genetically modified organisms). Genetic modification has created soybeans that are resistant to herbicides. When the herbicides are applied to kill the weeds, the soybeans are not affected. Genes for making vaccines have been put into plants, which can solve the problems of cost and global shortages of vaccines. Large databases have been developed to help find the genes necessary for these genetic modifications. The long term impact of these new processes is unknown. Have these new discoveries been properly scrutinized?

Diverse metabolic processes can be used to help us clean up our polluted planet. Some bacteria are used to break down oil spills, remove benzene from polluted waters, and eliminate toxic mercury. The recent discovery of groups of organisms called biofilms could be very important for these types of processes.

Biopharming uses genetically modified animals and plants to produce proteins for therapeutic use. DNA microarrays can be used to test for genetic predisposition to a disease. Viral vectors can be used in gene therapy. Much medical research relies on biotechnology and bioinformatics. Bioinformatic databases allow us to access vast quantities of information easily so that we can use them to understand how our genes function and how they make proteins that either keep us healthy or make us susceptible to disease.

NATURE OF SCIENCE

Serendipity has led to scientific discoveries: the discovery of penicillin by Alexander Fleming could be viewed as a chance occurrence.

B.1 Microbiology: organisms in industry

Understandings:

- Microorganisms are metabolically diverse.
- Microorganisms are used in industry because they are small and have a fast growth rate.
- Pathway engineering optimizes genetic and regulatory processes within microorganisms.
- Pathway engineering is used industrially to produce metabolites of interest.
- Fermenters allow large-scale production of metabolites by microorganisms.
- Fermentation is carried out by batch or continuous culture.
- Microorganisms in fermenters become limited by their own waste products.
- Probes are used to monitor conditions within fermenters.
- Conditions are maintained at optimal levels for the growth of the microorganisms being cultured.

Applications and skills:

- Application: Deep-tank batch fermentation in the mass production of penicillin.
- Application: Production of citric acid in a continuous fermenter by *Aspergillus niger* and its use as a preservative and flavouring.
- Application: Biogas is produced by bacteria and archaeans from organic matter in fermenters.
- Skill: Gram staining of Gram-positive and Gram-negative bacteria.
- Experiments showing zone of inhibition of bacterial growth by bactericides in sterile bacterial cultures.
- Skill: Production of biogas in a small-scale fermenter.

Microorganisms in industry

There are three main reasons why microorganisms are used in industry.

1. They are small. Microorganisms such as yeast and bacteria are single-celled organisms.
2. They have a fast growth rate. For example, bacteria reproduce by binary fission (splitting) and can reproduce in 30 minutes. If you start with 100 cells at time 0, how many cells will you have in 30 minutes? In 60 minutes? In 90 minutes? The answers: 200, 400, and 800, respectively.
3. They are metabolically diverse. This means that they have diverse sources of carbon, which they use to build other molecules. Some microorganisms use larger organic molecules such as glucose, $C_6H_{12}O_6$ for a carbon source. Others use molecules as small as methane, CH_4. They also use diverse sources of energy. Some microorganisms use sunlight and others use the energy held in the chemical bonds of molecules.

Bacterial colonies growing on nutrient agar in a Petri dish.

Microorganisms can be classified into four nutritional groups based on their type of metabolism.

- Photoautotrophic organisms use sunlight for energy, and carbon dioxide (CO_2) as their carbon source. Examples include algae.
- Photoheterotroph organisms use sunlight for energy, and carbon from organic compounds as their carbon source. Examples include purple bacteria.

- Chemoautotroph organisms use inorganic compounds for energy, and carbon dioxide as their carbon source. Examples include sulfur bacteria that use hydrogen sulfide (H_2S) for energy.
- Chemoheterotroph organisms use preformed organic compounds as their energy source and as their carbon source. Examples include fungi, protozoa, and bacteria.

CHALLENGE YOURSELF

1 Fill in Table 13.1 as you read about the metabolic diversity of microorganisms.

Table 13.1 Examples of different types of metabolism of microorganisms

Type of metabolism	Energy source	Carbon source	Example

'Auto' means self and 'troph' means feeder, so an autotroph is a self-feeder. An alga makes its own glucose through the process of photosynthesis. 'Hetero' means other. Bacteria must decompose other organisms or products of organisms in order to get their food.

Products made by microorganisms

What are some of the products made from microorganisms? See how many you can think of before you look at the lists of examples below.

Foods:

- bread
- cheese
- yogurt
- wine
- beer
- soy sauce
- many more.

Commodities:

- food additives such as amino acids and vitamins
- solvents such as alcohol and acetone
- biofuels such as ethanol and methane.

Chemicals:

- pharmaceuticals such as antibiotics and steroid hormones
- biochemicals such as enzymes and proteins.

In the making of yogurt lactose sugar in the milk is broken down by the lactase enzyme of the bacteria fermenting the milk.

Pathway engineering

Remember the enzymatic pathways you studied which are necessary for cell respiration and photosynthesis? Using pathway engineering, scientists attempt to introduce new genes to adjust these pathways. Pathway or metabolic engineering is the practice of optimizing genetic and regulatory processes within microorganisms for our use. The point of controlling the genes of a microorganism and regulating its biochemical pathways is to increase the production of a substance that we want by that cell.

Photosynthesis and respiration are examples of pathways. Microorganisms have certain pathways that they use to make a product that they need. Genetic engineers can change these pathways by giving the microorganism a new gene. With a new gene there is a new product, and we are interested in that new product.

Here is an example of pathway engineering.

- A bacteria such as *Escherichia coli* has a biochemical pathway that it uses to make a short-chain (2-carbon) alcohol.
- We introduce new genes into the *E. coli* bacteria that change the genes and modify the way the pathway works. In other words, we regulate the pathway by changing the genes that control the pathway.
- The product of the pathway is now a long-chain (5-carbon) alcohol made by the *E. coli*. The pathway has been engineered.

Such an engineered pathway was first achieved at UCLA at the Henry Samueli School of Engineering and Applied Science. But why do we want to make longer chain alcohols? Longer chain molecules are of interest to us because they contain more energy, and are important in the production of gasoline and jet fuel.

Metabolites of interest

Look back at the nutritional types of microorganisms. Notice that photoheterotrophs and chemoheterotrophs need organic molecules as their source of carbon. Most bacteria and yeast are either photoheterotrophs or chemoheterotrophs. Because glucose and glycerol are simple organic molecules, they are perfect as inexpensive carbon sources.

The alcohol produced by *E. coli* in the example above is called a metabolite. A metabolite is a product of a biochemical pathway. Enzymes regulate these pathways and genes control the enzymes. Thus we have seen with *E. coli* that a change in genes can affect the pathway and produce a desired product, the metabolite of interest. This can be done without interfering with the normal bacterial growth and reproduction. Industrial microbiology attempts to modify the existing pathways of microorganisms so that they can be efficient factories for particular compounds (the metabolites of interest). Pathway engineering has been very successful using bacteria and yeast because:

- these organisms have a high yield compared with plants
- these organisms have a fast growth rate
- the desired product can be easily purified
- the carbon sources needed (glucose or glycerol) are simple and inexpensive.

The beneficial outcomes of the technique of pathway engineering, which was only developed in the 1990s, include:

- sustainable processes for the production of fuel and chemicals from renewable sources
- drugs to treat diseases
- increased production of antibiotics and supplements
- processes to help clean up the environment.

Successful pathways

The French company Sanofi has begun brewing baker's yeast to make malaria drugs on an industrial scale. It will produce 70 million doses a year. This breakthrough was published in the journal *Nature* in April 2013. The drug is called artemisinin.

Development of new drugs to fight diseases such as malaria is one of the benefits of pathway engineering.

Malaria affects millions of people and kills 650 000 a year. Most victims are children.

Malaria is one of the oldest diseases known to humans. In the 4th century BC malaria devastated the populations of the Greek city states.

Amyris is the biotech start-up company that initially engineered the pathway to produce artemisinin. Before that, the drug could only be obtained from sweet wormwood plants. The costs of obtaining it from the plants are high and production is unstable. Three enzymes were isolated and taken from the sweet wormwood plant and introduced into a pathway in baker's yeast. The pathway-engineered process will take about 3 months to produce the metabolite of interest, compared with the 15 months for the plant-based method. The pharmaceutical company Sanofi has pledged to sell the drug without profit.

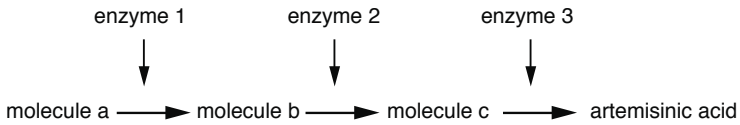
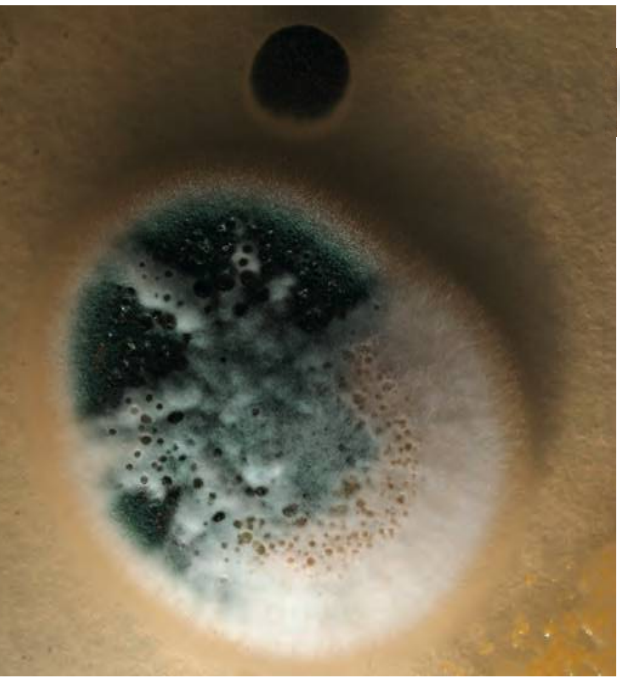


Figure 13.1 The pathway to artemisinic acid.

Figure 13.1 shows the pathway to artemisinic acid, which will be chemically converted into the malaria drug artemisinin. The enzymes are obtained from the sweet wormwood plant.

Researchers at MIT and Tufts University have engineered a metabolic pathway of the bacteria *E. coli*. They have enabled it to produce a large quantity of a precursor molecule to the important anti-cancer drug Taxol. Taxol is a powerful inhibitor of cell division that is used to treat ovarian, lung, and breast cancer. This drug was initially isolated from the Pacific yew tree, *Taxus brevifolia*. Two to four trees are needed to produce enough drug to treat one patient. Drug companies anticipate that using *E. coli* will significantly lower the cost of the drug.

Half of all prescription drugs are from rainforest plants or marine sponges. Sweet wormwood comes from the forests of China. However, rampant deforestation is decimating China's forests: 80% have already been lost.



Colony of *Penicillium* mould growing in a Petri dish of nutrient agar. Notice the shiny yellow bacterial colonies in the lower right corner. The mould may just grow over the top of the bacteria and kill the colonies.

Figure 13.2 A fermenter. http://www.htl-innovativ.at/index.php?lang=eng&modul=detail&id=178

Fermentation

NATURE OF SCIENCE

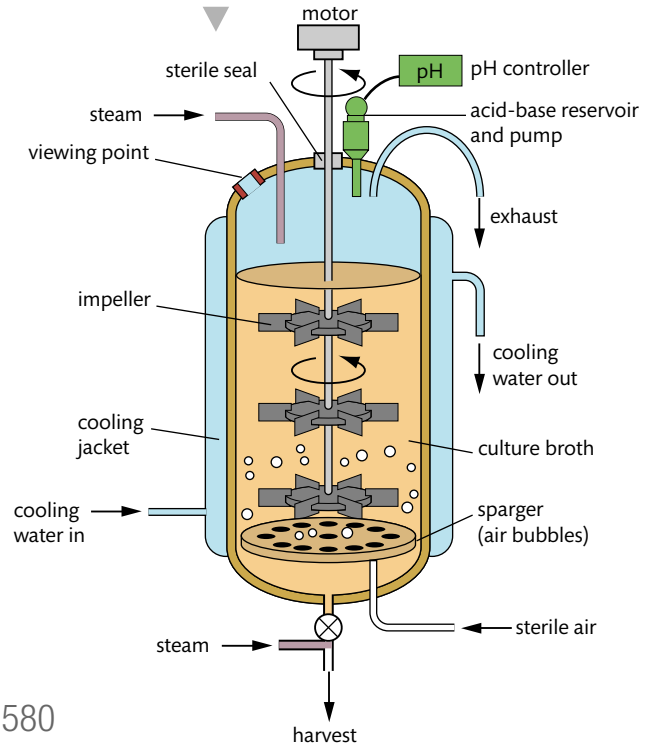
The discovery of a bacteria that causes stomach ulcers was the result of serendipity. The researchers who hypothesized that it was not acid but bacteria that caused ulcers could not get the bacteria to grow. Without evidence, there was no support for their hypothesis. By accident they left the bacteria in a culture dish for 4 days over a holiday weekend. When they returned the bacteria had grown. In 1950, J. Robin Warren and Barry Marshall won the Nobel Prize for this discovery. Antibiotics can often be used to cure ulcers instead of surgery.

In 1928, Alexander Flemming, a Scottish biologist, noticed that *Penicillium notatum*, a mould, had killed staphylococcus bacteria in a culture dish. It was then discovered that penicillin has an active ingredient that inhibits the synthesis of cell walls of bacteria, so preventing them from reproducing. That initial serendipitous discovery let to the development of penicillin as an antibiotic. Penicillin was used to treat the thousands of wounded during World War II, thus saving many lives.

TOK

As it is possible to come up with many hypotheses to fit a given set of observations, how did Alexander Flemming in 1928 prove that it was the mould that had killed the staphylococcus bacteria in the culture dish, and not something else?

Industrial microbiology is now growing microorganisms on a large scale to produce valuable products such as penicillin commercially. This process is referred to as fermentation. Currently, antibiotics are the most important product of fermentation.



Large-scale production of metabolites

The need for penicillin means that there is a demand for large-scale production. Fermenters have been developed that are large-scale vats that can be controlled so that fermentation can take place in an optimal environment. Fermenters have:

- a size that fits the need for optimum production of the desired metabolite, e.g. penicillin
- a means of mechanical agitation or air bubbles for mixing the microorganism with the substrate materials
- devices to maintain the optimum temperature
- probes to monitor the environment for optimum industrial production
- processes for avoiding contamination.

Eventually, the end product can be turned into crystals, packaged, and sold.

Probes

Sterile probes are commonly used to monitor the following conditions in large-scale fermenters:

- oxygen concentrations
- carbon dioxide concentrations
- pH levels
- temperature
- pressure
- stirrer speed.

An imbalance in the any of above could have a harmful effect on the growth of the microorganism that is producing the metabolite of interest.

This is *Penicillium* again growing in a Petri dish. Remember that when the mould is starving it makes penicillin. In batch fermentation, we need to duplicate the starvation mode so that the mould will make large quantities of penicillin.

Batches

A batch is the volume of nutrients and other materials (substrate) added to a fermenter. Two types of batches are:

1. fed-batch, where the nutrient and substrate are added a little at a time
2. continuous-batch, where the substrate is added continuously and an equal amount of fermented medium is continuously removed.

Deep-tank fermentation of penicillin

The current type of mould that is used to produce penicillin industrially is *Penicillium chrysogenum*. This mould gets its energy to reproduce and grow from glucose. This occurs in a liquid medium in flasks outside the batch fermenter, and produces pyruvic acid as the primary metabolite. We do not want to collect this metabolite, but its production is unavoidable because it is a product of the mould breaking down glucose to get energy. When the biomass of *Penicillium* has reached a sufficient level, it is placed in the batch fermenter (see Figure 13.3).

The batch fermenter is missing glucose, which will starve the *Penicillium*. Why do we want this mould to starve? It is in fact when *Penicillium* mould is starving that it makes penicillin! Penicillin is a secondary metabolite produced in times of stress by the *Penicillium* mould: it is a defence mechanism against other organisms in its environment. Secondary metabolites are metabolites produced by a microorganism that are not used for energy. We need to duplicate the starvation mode for the mould so that it will make large quantities of penicillin.

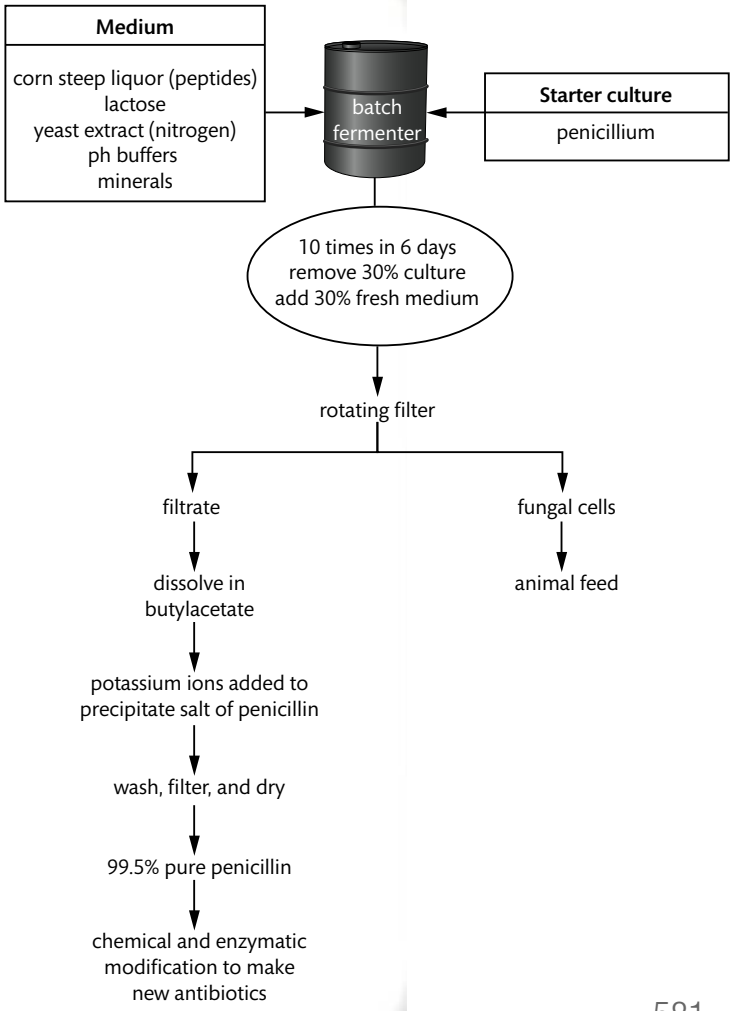


Figure 13.3 The fed-batch production of penicillin.

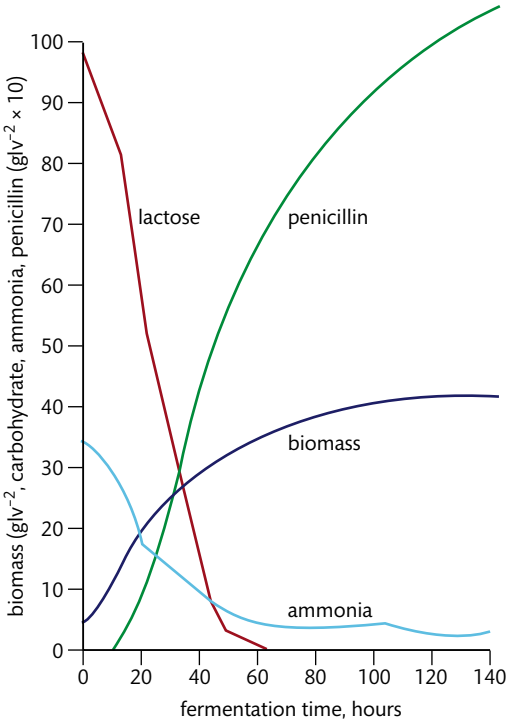


Figure 13.4 Penicillin fermentation using *Penicillium chrysogenum* during secondary metabolism. The production of penicillin increases as the biomass of the mould levels off (the stationary phase).

The following substances are put into the deep-tank fermenter to produce penicillin:

- lactose
- yeast extract
- corn steep liquor
- buffers
- minerals.

Using lactose in the medium of the batch fermenter will begin to starve the *Penicillium*. Notice on the graph that, as the lactose is broken down, the penicillin is produced. Yeast extract is a source of nitrogen; corn steep liquor provides peptides; buffers resist pH changes; and minerals are needed by the mould for nutrients.

Also notice on the graph that the biomass of the mould is levelling off while the penicillin production is increasing. The stage of bacterial growth when penicillin is produced is called the stationary phase. The bacteria is hardly reproducing at all, but making large quantities of penicillin. Why?

The mould is in a stressful situation because of the lack of a sugar and carbon source. It responds by making penicillin to defend itself against other organisms that might be present and competing with it for the lactose.

Optimal conditions in the fermenter

Optimal conditions in a deep-tank fermenter are maintained by:

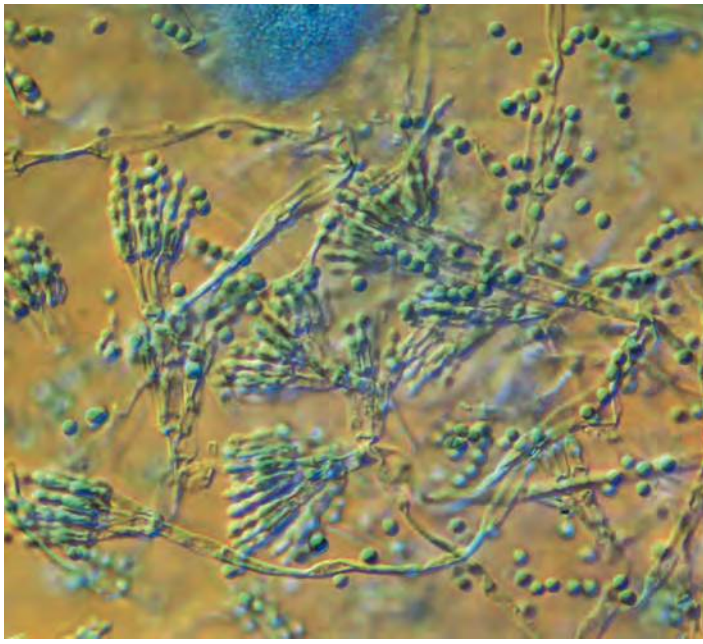
- a fed-batch method, which is ideal to keep *Penicillium* producing penicillin
- probes, which measure the pH, temperature, and oxygen levels
- oxygen, which is added by the sparger (see Figure 13.2) because *Penicillium* is an aerobic organism and needs an oxygen supply for fermentation
- a cooling jacket, which reduces the heat given off by metabolism
- NaOH, to maintain the correct pH of 6.5.

Fermenters are limited by their own waste products

When penicillin builds up in the fermenter, the excess penicillin inhibits an enzyme in the penicillin-producing pathway, so production stops. Thus the penicillin product must be removed efficiently for the system to continue. The volume in the fermenter must remain constant, so more material is added in a fed-batch manner as the product is removed.

Continuous-batch fermentation of citric acid

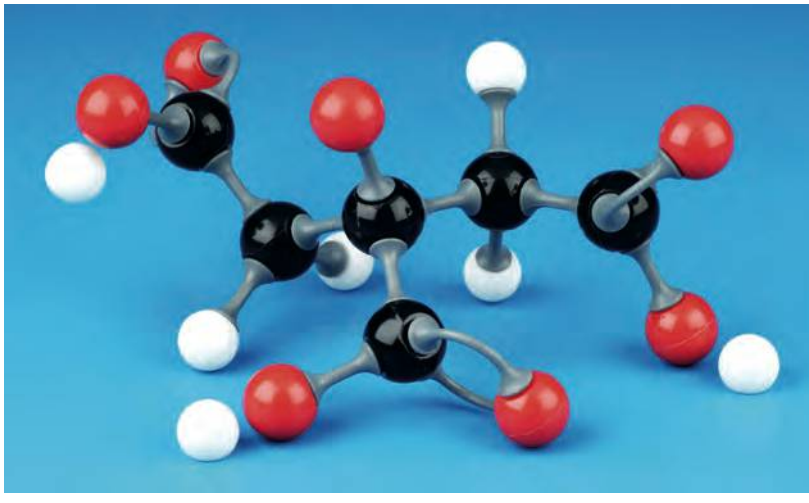
Another product that is very commonly used and made by a mould is citric acid. It is not an antibiotic but a food additive. Look at a can of tomatoes in your food cupboard and you will probably see citric acid on the label. You might also see citric acid on the ingredient lists of powdered drinks, jars of jam, jars of maraschino cherries or sundried tomatoes, and many other foods. Citric acid is one of the most important industrial microbial products: 550 000 tons of citric acid are made every year by the simple mould *Aspergillus niger*.



Aspergillus is a common species of mould found all over the world in many different climates. This mould grows on carbon-rich substrates such as glucose and starch.

Uses of citric acid

Before the production of citric acid by fermentation, it was obtained from the juice of citrus fruit. When World War I interfered with the harvesting of the Italian lemon crop, natural citric acid became a rarity. In 1917 an American food chemist discovered that *A. niger* could efficiently produce citric acid. Industrial production was started 2 years later. Citric acid is a flavour enhancer, maintains the pH of a food product, and can be used as a preservative. Most industrially produced citric acid is made using *A. niger* with molasses as the substrate, i.e. as the carbon and sugar source.



In this molecular model of citric acid carbon is shown in black, hydrogen in white and oxygen in red.

Production of citric acid

Researchers have found that continuous-batch fermentation for 50 days using molasses as a substrate gives an 85% yield of citric acid, whereas fed-batch fermentation only yields 65% of citric acid.

Continuous-batch fermentation is an open system where equivalent amounts of a sterile nutrient solution such as molasses are added to the fermenter. An equal amount of solution containing the metabolite of interest is withdrawn. Thus the total amount remains the same. This maintains a steady-state in the fermenter, where the loss of mould cells is balanced by the growth of new mould cells.

CHALLENGE YOURSELF

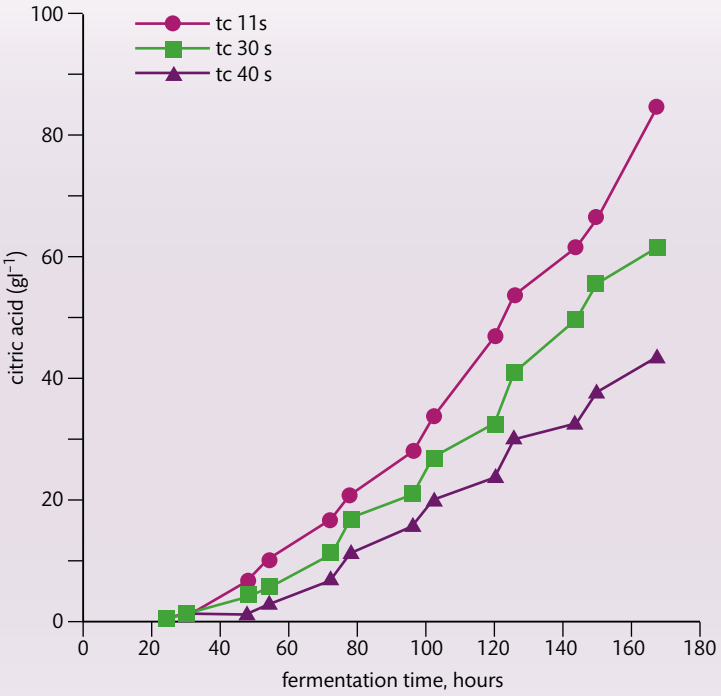


Figure 13.5 The effect of agitation: the relationship between circulation time (tc) and citric acid production by *A. niger* in a tubular loop bioreactor. Papagianni 2007

2 Look at Figure 13.5. Compare and contrast the results of agitation time on citric acid production at 11 s, 30 s, and 40 s.

3 What conclusion can you draw from this graph?

4 Formulate a hypothesis as to why this is occurring.

Biogas production by archaeans and bacteria

One of the renewable energy sources of the future may be biogas. In the UK it is projected that 17% of vehicle fuel has the potential to be replaced by compressed biogas. Biogas can be used for heating and cooking as well as running engines. Where does biogas come from and how do we get it? Not surprisingly, it is one more product that can be produced by microorganisms.

Classification of archaeans

Carl Woese was studying microorganisms when he realized that scientists were making mistakes in their classification of living things. Thanks to new technology, Woese and his colleagues noticed a large difference in the ribosomal (r)RNA of a group previously considered to be prokaryotes. Based on this, Woese and his colleagues suggested a classification level called a domain. According to this system, there are three domains of all living things: Archaea, Eubacteria (prokaryotes), and Eukaryote (eukaroytes).

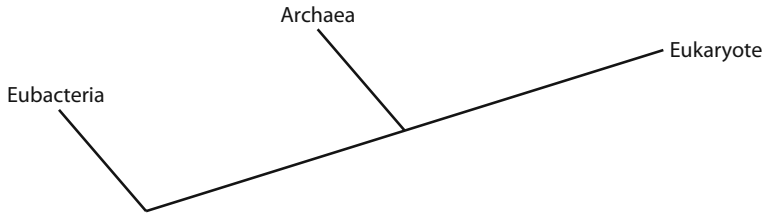


Figure 13.6 The three domains for classifying living organisms.

- Eubacteria: 'true' bacteria, prokaryotes with no organized nucleus and no membrane-bound organelles. An example is *Escherichia coli*, which is commonly found in animal waste products.
- Archaea: archaeabacteria or 'ancient' bacteria are also prokaryotes. Most groups live in extreme environments. An example is the sulfur bacteria that inhabits the hot springs of Yellowstone National Park in the USA.
- Eukaryote: single-celled and multicellular organisms that all have their DNA contained in a nucleus. The kingdoms of plants, animals, protists, and fungi belong here.

Biogas fermenter

Both prokaryotes and archaeans work in a fermenter when biogas is produced. In the fermenter, the enzymes of the prokaryotes and archaeans break down biodegradable materials such as plant products by anaerobic digestion (digestion without oxygen). The materials produced are simple molecules, one of which is biogas. Biogas is made of:

- 50–75% methane
- 25–45% carbon dioxide
- 0–10% nitrogen
- 0–3% hydrogen sulfide
- 2–7% water.

In a biogas fermenter the following process takes place, in this sequence, in the absence of oxygen:

1. liquefaction, the hydrolysis (splitting) of long-chain organic compounds
2. acidification, resulting in short-chain fatty acids, plus hydrogen and carbon dioxide
3. acetic acid formation, resulting in acetic acid, plus hydrogen and carbon dioxide
4. methane formation (methanogenesis), the action of archaean bacteria on the products to produce methane.

Each of the four stages requires specific bacteria. In the fourth stage, the microorganism required to produce the biogas (methane) is an archaean. Other factors must be kept constant:

- there must be no free oxygen (the bacteria in the fermenter are anaerobic)
- the temperature must be about 35°C
- the pH must not be too acidic because methane-producing bacteria are sensitive to acid.

Sometimes small farms use biogas fermenters. The biogas produced can be used to run electrical machinery, which reduces a farmer's costs. A high-quality fertilizer without weeds or odour is a by-product that can be used instead of manure, and

Large biogas fermenters: the advantage of biogas over wind and solar is that it is always available. Biogas can be stored and accumulated.

Using a small-scale biogas digester such as this one in India, farmers can trap methane instead of letting it escape from rotting manure into the environment. Methane from farms is a significant greenhouse gas.

pollution caused by water run-off containing animal waste is reduced. Globally, methane emission is lowered with the use of biogas. Methane is a greenhouse gas that contributes to global warming.

Gram staining

In order to help identify different groups of bacteria, staining techniques are used. Bacteria can be divided into two groups based on the structure of their cell wall; the Gram stain differentiates between the two types. Gram-positive bacteria have a simple cell wall and Gram-negative bacteria have a cell wall that is more complex (see Figure 13.7). They differ in the amount of peptidoglycan present. Peptidoglycan is an important material for bacteria. It consists of sugars joined to polypeptides, and acts like a giant molecular network protecting the cell. Gram-positive bacteria have large amounts of peptidoglycan and Gram-negative bacteria have a small amount. Only Gram-negative bacteria have an outer membrane with attached lipopolysaccharide molecules. Lipopolysaccharides are carbohydrates bonded to lipids. These molecules are usually toxic to a host. The outer membrane protects against the host defences. The outer membrane also protects the Gram-negative bacteria from antibiotics.

Can you see why an antibiotic like penicillin works more effectively against Gram-positive bacteria? The Gram-positive bacteria have no outer membrane to protect them from an antibiotic.

Table 13.2 compares the cell wall structure of Gram-positive and Gram-negative bacteria.

Table 13.2 Gram-positive and Gram-negative bacteria

	Gram-positive bacteria	Gram-negative bacteria
Cell wall structure	Simple	Complex
Amount of peptidoglycan	Large amount	Small amount
Peptidoglycan placement	In outer layer of bacteria	Covered by outer membrane
Outer membrane	Absent	Present with lipopolysaccharides attached

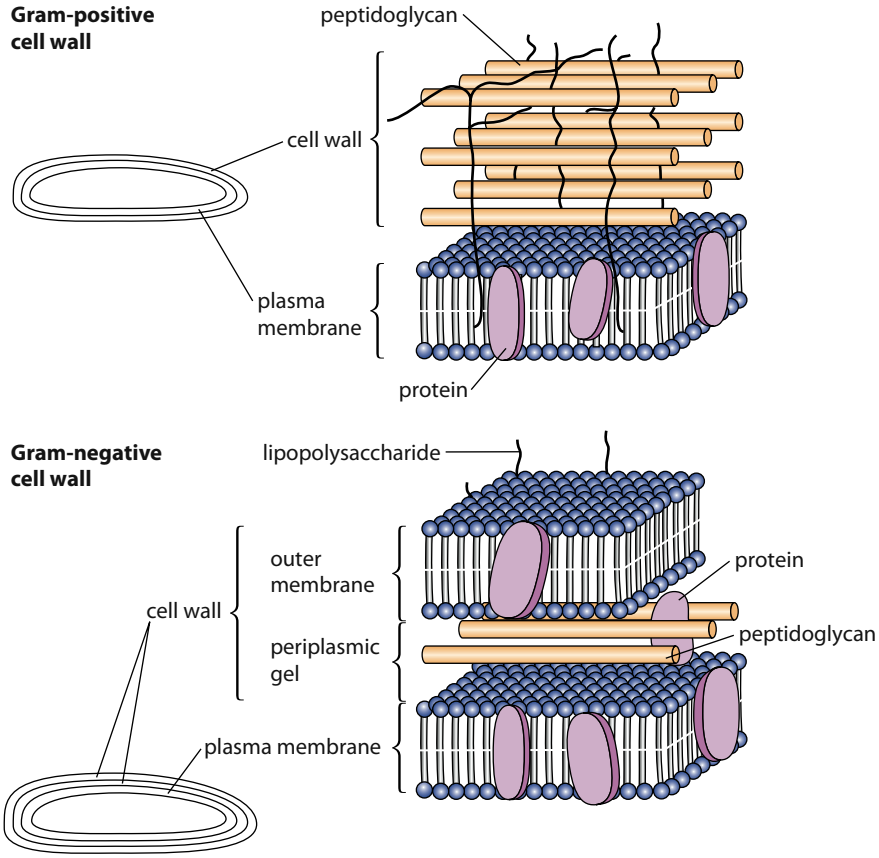


Figure 13.7 Diagram of the cell wall structure of bacteria.

Gram-stain technique

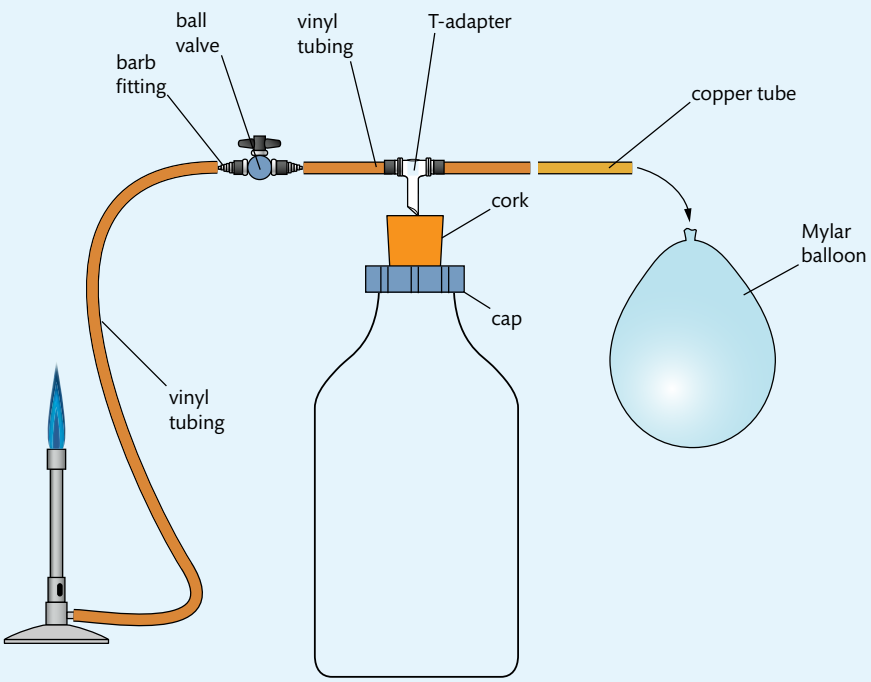
Safety alerts: Follow standard safety protocols for bacterial work.

Gram-positive bacteria retain the primary dye and Gram-negative bacteria are easily decolourized.

- Add bacteria to a glass slide and fix on the slide with heat.
- Apply crystal violet stain.
- Flood with iodine.
- Rinse off iodine.
- Decolorize with alcohol.
- Counterstain with safranin.

Gram-positive bacteria will stain violet and Gram-negative will stain pink.

Production of biogas in a small-scale fermenter



When a slurry of organic material and water is added to the fermenter, eventually methane will be produced. The methane can be collected in the Mylar balloon. To test whether it is methane, attach the burner and squeeze the balloon. If the burner lights it is methane gas rather than just carbon dioxide.

Figure 13.8 Production of biogas in a small-scale fermenter.

Biogas may be directly combustible and used in boilers, turbines, or fuel cells. It can be used for heating water, producing steam, or for space heating. Biogas can be used in all applications designed for natural gas.

Exercises

1. Describe pathway engineering.
2. Compare and contrast batch and continuous culture.
3. Compare and contrast Gram-negative and Gram-positive bacteria.
4. List four reasons why pathway engineering of bacteria and yeast has been very successful.

NATURE OF SCIENCE

Assessing risks and benefits associated with scientific research: scientists need to evaluate the potential of herbicide resistance genes escaping into the wild population.

B.2 Biotechnology in agriculture

Understandings:

- Transgenic organisms produce proteins that were not previously part of their species' proteome.
- Genetic modification can be used to overcome environmental resistance to increase crop yields.
- Genetically modified crop plants can be used to produce novel products.
- Bioinformatics plays a role in identifying target genes.
- The target gene is linked to other sequences that control its expression.
- An open reading frame is a significant length of DNA from a start codon to a stop codon.
- Marker genes are used to indicate successful uptake.
- Recombinant DNA must be inserted into the plant cell and taken up by its chromosome or chloroplast DNA.
- Recombinant DNA can be introduced into whole plants, leaf discs, or protoplasts.
- Recombinant DNA can be introduced by direct physical and chemical methods, or indirectly by vectors.

Applications and skills:

- Application: Use of tumour-inducing (Ti) plasmid of *Agrobacterium tumefaciens* to introduce glyphosate resistance into soybean crops.
- Application: Genetic modification of tobacco mosaic virus to allow bulk production of Hepatitis B vaccine in tobacco plants.
- Application: Production of Amflora potato (*Solanum tuberosum*) for paper and adhesive industries.
- Skill: Evaluation of data on environmental impact of glyphosate-tolerant soybeans.
- Skill: Identification of an open reading frame (ORF).

Guidance

- *A significant length of DNA for an open reading frame contains sufficient nucleotides to code for a polypeptide chain.*
- *Limit the chemical methods of introducing genes into plants to calcium chloride and liposomes.*
- *Limit the physical methods of introducing genes into plant to electroporation, microinjection, and biolistics (gunshot).*
- *Limit vectors to* Agrobacterium tumefaciens *and tobacco mosaic virus.*

Genetic modification of crops

You may have already heard of genetically modified (GM) crops. A GM plant has been modified with the introduction of a gene that does not normally occur in that species. When genes are expressed, the result is a protein or series of proteins. GM plants have been given new genes so that new proteins are made.

GM soybeans growing in a field. Debate about genetically modified food is raging. The opponents of GM plants object to the transfer of genes to another species. Proponents argue that GM plants will increase crop yield and help us feed 9.2 billion people. What is your opinion? What are the facts that support your opinion?

A new proteome

A proteome is the set of proteins expressed by the genome (all the genes) of a species. For example, soybeans have certain genes that express proteins that give the soybean specific traits. Proteins can be enzymes or structural molecules that cause physical characteristics (e.g. colour and leaf shape). When a new gene is introduced into a species, that new gene is called a transgene. Transgenic organisms produce proteins that were not previously part of their species' proteome. For example, a gene can be introduced to make soybeans resistant to the herbicide glyphosate. When glyphosate is sprayed onto weeds, the transgenic soybean is not harmed because it is resistant to

In the 1800s, Thomas Malthus predicted that food demand would outstrip food supply.

it. The weeds are killed and the soybean crop benefits. The transgenic soybeans are an example of a genetically modified organism (GMO).

Increasing crop yield

Why do we need biotechnology to help increase crop yields? By 2050 the world population will have increased to 9.2 billion, a 4-fold increase in 100 years, making food production a huge social issue. In the 1800s Thomas Malthus famously predicted that our food demand would outstrip our food supply. We are facing that situation now. However, new technologies using recombinant DNA to produce transgenic crops may be able to increase the yield of some of the basic crops. In 2007, 12 million farmers in 23 countries were growing GM crops.

Environmental resistance

The goal of GM crops is to overcome environmental resistance to increase crop yields. Environmental resistance consists of limiting factors in the environment which keep populations from reaching their maximum growth potential. Introducing a new gene can enhance the capacity of a crop plant to overcome the limitations of their environment. Some examples of limitations to crop yield and how they have been overcome are as follows.

- Insects: GM plants resistant to insects give a higher yield; examples of such GM crop plants include tobacco, tomato, potato, cotton, maize, sugar cane, and rice.
- Viral disease: 20 plant species are resistant to 30 viral diseases, preventing huge crop losses; for example, papaya has been given a gene that helps it resist the ring spot virus.
- Weeds: when a herbicide is sprayed to kill weeds, herbicide-resistant plants are not harmed and so the crop is not affected; for example the crop yield of GM soybeans is higher.
- Drought: drought resistance can help prevent crop damage; for example, rice has been engineered so that it is protected against prolonged drought.

GM plants can overcome these factors that limit crop yields.

By 2007 Spain was producing 50% of GM crops. Spain is the major European producer of maize genetically modified with a pesticide gene that kills insects that attack it. China is increasingly producing cotton with the same pesticide gene: currently 66% of its cotton crop has this gene.

Fifteen per cent of post-harvest food crops in developing countries is lost to insects.

Novel products from GM plants

Novel products from GM plants include vitamins, pharmaceuticals, enzymes, and vaccines. Below are specific examples of the outcomes of genetic modification. As you will see, the introduction of new genes into crop plants can be done by physical methods, chemical methods, or by using a microorganism as a vector. Two microorganisms that are commonly used are a bacterium, *Agrobacterium tumefaciens*, and a virus, the tobacco mosaic virus, TMV.

Glyphosate resistance in soybean plants

Using less pesticide and herbicide is a goal. It was recognized in the 1950s that herbicides and pesticides harm many other organisms in an ecosystem as well as the targets. The development of herbicide-resistant soybeans has been developed as a response to this concern. Using a bacterium that naturally infects plants as a vector, a herbicide-resistant gene has been introduced into soybeans, *Glycine max*.

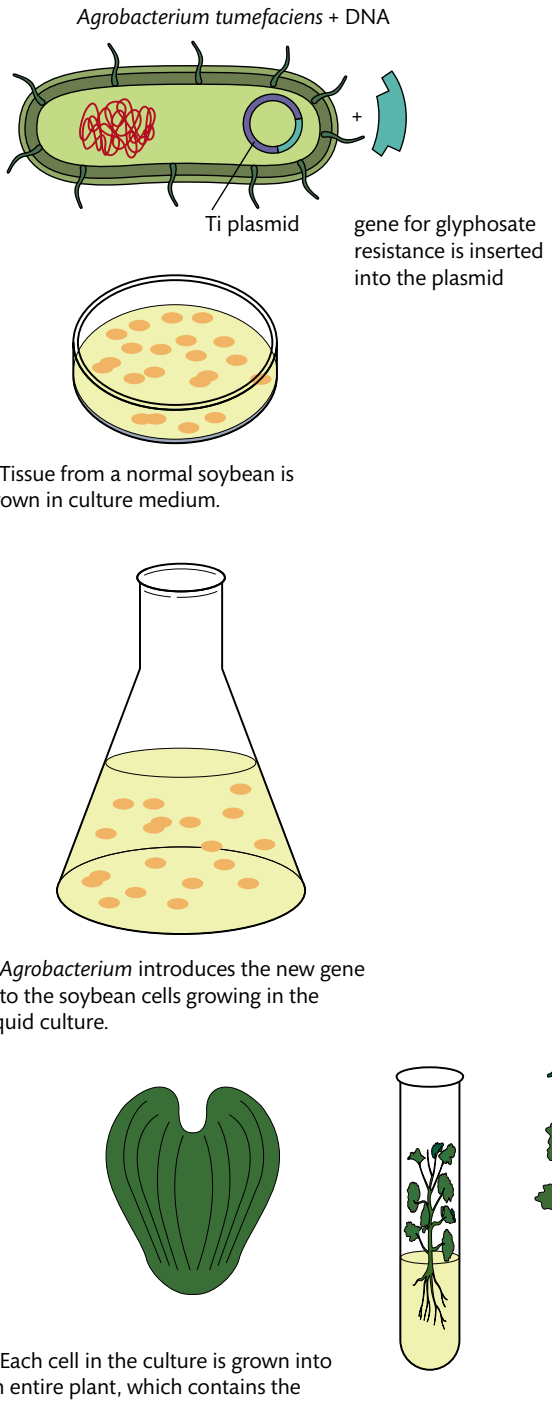


Figure 13.9 *Agrobacterium* is used to introduce glyphosate resistance into soybeans, *Glycine max*.

Agrobacterium tumefaciens (agrobacter) is a pathogenic (disease-causing) bacterium that attacks plants. It can be engineered to be non-pathogenic but still have the ability to insert DNA in to a plant. Agrobacter contains a circular piece of DNA called a plasmid that can enter a plant cell and insert genes into its chromosome. Scientists have developed methods to engineer this plasmid, called a Ti plasmid (tumour-inducing plasmid), and make it a vector for carrying genes of interest into plants. The plants express the gene by making a protein that is the desired product. In the case of soybeans, the protein is an enzyme that allows the plant to use an alternative pathway

that causes resistance to the herbicide glyphosate. We call the soybeans glyphosate-tolerant soybeans. The common name for glyphosate is Roundup. Plants that contain this herbicide are called 'Roundup ready'. Fields can be sprayed with glyphosate and the weeds are killed but the soybeans are not affected. Glyphosate is a broad-spectrum herbicide that travels in the phloem of the plant and is readily translocated to roots, stems, and leaves. It inhibits an enzyme, EPSPS, that is necessary for making essential amino acids. Without these essential amino acids, a plant cannot synthesize the proteins needed for growth.

Soybeans are a very valuable crop. An enormous amount of protein is produced per acre by soybeans. Soybean products include tofu, soymilk, and soy sauce.

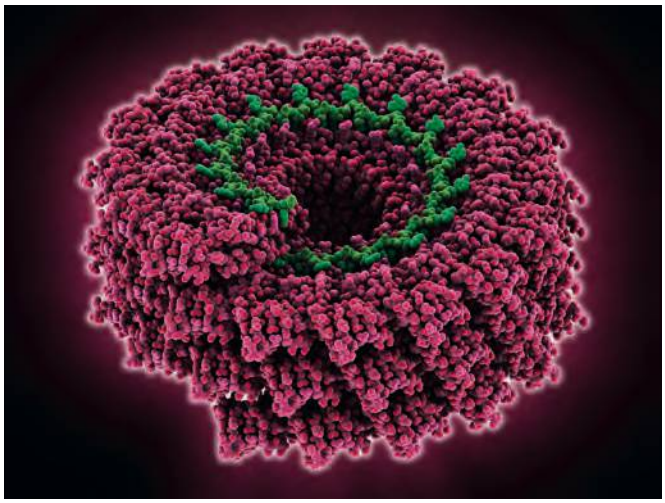
Hepatitis B vaccine production from tobacco plants

Hepatitis B is an infectious disease of the liver caused by the hepatitis B virus. The disease has caused epidemics in many parts of the world. Vaccines for this disease have been routinely used since the 1980s. For years this vaccine has been made from yeast, but it is not cheap and has to be refrigerated. Most developing countries cannot afford it.

Hepatitis B is a vaccine that can be made by tobacco plants in bulk. A gene that makes an antibody to hepatitis B is inserted into a modified version of the tobacco mosaic virus (TMV). TMV is a retrovirus that has the capacity to cause disease in tobacco plants. As the virus is scratched on to the leaves of the tobacco plant, the plant becomes infected with the gene-carrying virus. The virus transfers the gene to the plant cells, and the result is the generation of antibodies. After a few days, leaves can be cut and vaccine collected. Tobacco plants have plenty of biomass, so it is easy to see how bulk vaccines can be made.

NATURE OF SCIENCE

In order to make sure that the plasmid has transferred to the agrobacter cell, agrobacter is grown on culture media containing an antibiotic. In the circular plasmid, Figure 13.9, that there is a 'gene for antibiotic selection'. If the plasmid has been transferred, then the agrobacter will grow in the presence of the antibiotic. If no plasmid is present, the antibiotic will kill the agrobacter. This test provides data confirming the hypothesis that the plasmid has successfully transferred to the bacteria before it is put into the tobacco plant. Scientists must constantly collect data to support the hypotheses that they are formulating.



Computer model showing the molecular structure of the tobacco mosaic virus (TMV). This virus is made of RNA (green) and a protein coat (pink).

The Amflora potato

Just recently, for the first time since 1998, a GM crop has been approved to be grown in a European Union (EU) country. BASF Plant Science has developed a genetically modified potato, *Solanum tuberosum*, plant that is not to be consumed as a food product but to be used by industry. In order to be approved, various safeguards have been put in place to prevent this potato from mixing with conventional potato plants. Many rules and regulations must be followed about where the crop is grown, who grows it, and how it is shipped to a factory.

The potato is called the Amflora potato, and it is a breakthrough in production of amylopectin, a type of starch made by potatoes. Normally, potatoes produce 20% amylose and 80% amylopectin. The Amflora potato produces 100% amylopectin, which is a desirable product for industry. The gene in this potato that produces the 20% amylose has been turned off. Amflora starch is beneficial to the paper and adhesive industry. It gives printer paper a glossier look and makes concrete stick better to walls.

Amflora is a genetically optimized potato that produces only one starch component and is used for technical applications.

NATURE OF SCIENCE

Scientists must assess the risks and benefits associated with scientific research. Genetic modification of crops has many risks to be considered:

- the potential for herbicide-resistance genes to escape into the wild population
- unintended harm to other organisms, such as insect pollinators and amphibians
- reduced effectiveness of herbicides
- possible human health risks, for example some studies have found glyphosate in human urine.

Have there been allergic reactions to the new gene put into a plant?

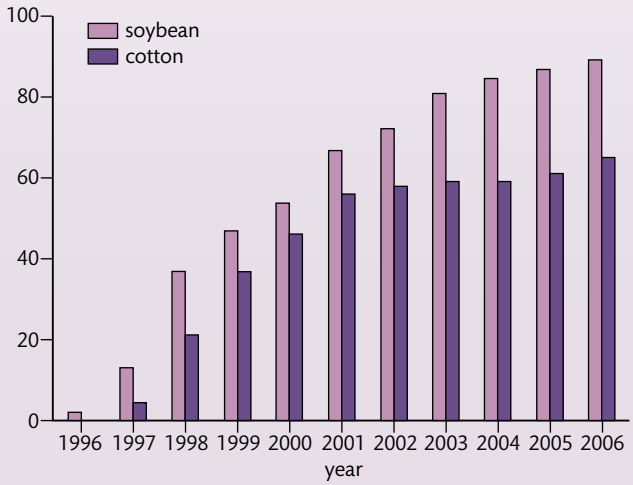
Despite regulatory approval by the EU, on 16 January 2012 BASF announced that it is pulling its genetic engineering division out of Europe and stopping production of its GM Amflora potato for the European market. The reason cited was lack of acceptance of this technology by consumers, farmers, and politicians

CHALLENGE YOURSELF

Adoption rates of GR (glyphosate-resistant) soybeans and cotton in the USA are shown in Figure 13.10. This bar chart shows the percentage of crop adoption over a 10-year period. Look at the bar chart and answer the following questions.

5 Compare and contrast the data regarding the two plant species.

6 Suggest a reason that might explain the differences.



NATURE OF SCIENCE

Are the risks worth it? Use the hotlinks at the end of this section to watch a movie called GMO/OMG that premiered in New York City in September 2013.

Figure 13.10 The percentage of soybean and cotton crop adoption over 10 years. Duke and Cerdeira 2007, Fig. 1

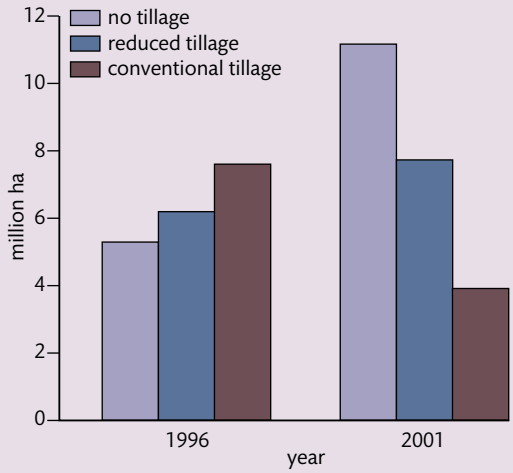


Figure 13.11 Soybean tillage methods by hectares farmed in the USA in 1996 and 2001. Duke and Cerdeira 2007, Fig. 2

Topsoil loss caused by tillage (the preparation of soil by mechanical agitation, such as digging, stirring, and overturning), is the most destructive effect of crops planted in rows. Tillage contributes to soil erosion by water and wind, soil moisture loss, and air pollution from dust. Glyphosate-resistant plants reduce tillage. Reduction in tillage improves soil structure, and results in reduced run-off and less pollution of rivers and streams.

Look at Figure 13.11 and answer the following questions.

7 Compare and contrast tillage results from 1996 and 2001.

8 Suggest a reason for these numbers.

9 Explain the environmental impact of these numbers.

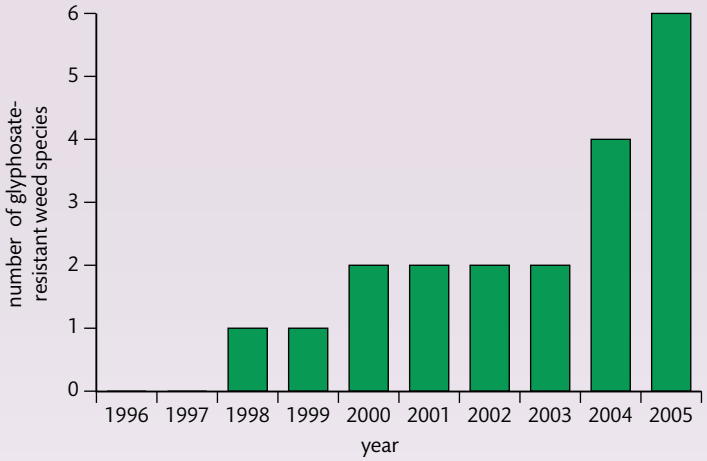


Figure 13.12 Glyphosate-resistant weed species in the USA.

Based on data from 'Facts About Glyphosate-Resistant Weeds', Purdue Extension, www.ces.purdue.edu/extmedia/GWC/GWC-1.pdf.

10 Describe the resistance seen in weed species in the USA to glyphosate.

11 Using the knowledge you have gained about how organisms change over time, describe how this may have occurred.

12 Compare and contrast resistance of weed species from 1996 to 2005.

13 Do some research and find one solution that scientists might suggest in solving this problem. Give one answer, although there may be many.

The word 'compare' in a question means you need to write down the similarities and contrast the differences between two or more things.

Discuss the view of Karl Popper that, for science to progress, scientists must question and criticize the current state of scientific knowledge.

Physical methods as a direct means of inserting genes into plants

In order to produce GM plants, methods had to be developed to deliver the transgene without damaging the plant cell. After introducing the gene, the plant cell must be able to reproduce an entire plant. The three methods used currently are: electroporation, microinjection, and biolistics. Just as one screwdriver does not work for all DIY jobs, molecular biologists have several tools to choose from as they try to transfer genes into a plant.

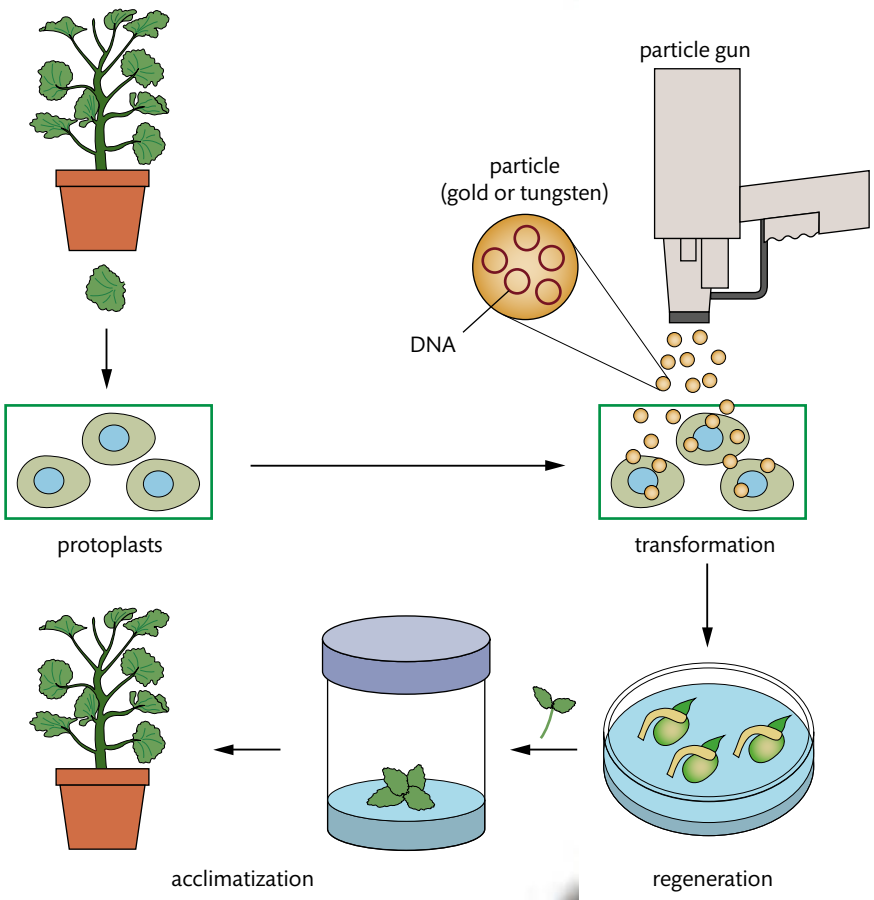
Electroporation

Electroporation makes pores in the cell membrane using electrical impulses. The cell membrane of a plant cell is surrounded by a cell wall that, as you will remember, is made of cellulose. The cell wall gives the cell shape. The cell wall is removed to expose the protoplast, a plant cell that has had its cell wall removed. When short high-voltage electrical impulses are applied to a suspension of protoplasts, small microscopic pores are created in the cell membrane, enabling DNA to enter the cell and nucleus. In this way, transgenes can be embedded in a plant cell.

Figure 13.13 Biolistcs (gunshot).

Biolistics

As you can see from Figure 13.13, with biolistics DNA is coated onto microparticles of gold or tungsten and fired with an explosive charge from a particle gun. The plant cells are transformed, meaning that the new DNA of interest is added to the chromosome of the plant cell. Finally, the transformed plant cell acclimates and regenerates into a plant.



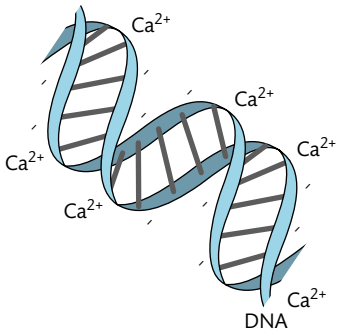
A gene gun or biolistic particle delivery system is a device for injecting cells with genetic information.

Electroporation and microinjection use protoplasts, which are plant cells without the cell wall. Biolistics coats particles with DNA and fires them right through the cell wall.

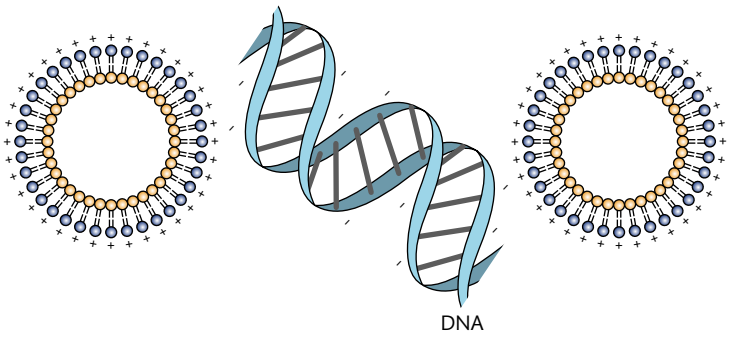
Microinjection

As the term suggests, with microinjection the DNA is injected into a protoplast with a microneedle. This method is very labour intensive because it is done one protoplast at a time. A whole plant is then grown from each protoplast.

Chemical methods as a direct means of inserting genes into plants



Calcium phosphate



Artificial liposomes

Figure 13.14 Transfection: DNA transfer in to a eukaryotic cell. Calcium binds with DNA for easy transfer into a cell; a liposome can also be used to carry DNA into a cell.

There are two chemical methods for inserting new DNA into plant cells.

The first method uses transfection. Transfection means to 'infect with a new DNA molecule'. With this procedure, calcium chloride and a buffered saline solution containing phosphate ions are added to the DNA. The calcium and phosphate ions bind with the DNA. Cells then take up the DNA by endocytosis or phagocytosis. This method is successful because the calcium phosphate coats the negatively charged DNA and neutralizes it. This allows the DNA to cross the cell membrane.

The second method uses liposomes. Liposomes are artificially prepared sacs of lipid molecules that have an aqueous interior. DNA can be put into the aqueous centre of the liposome. Next the liposome is fused with the lipid bilayer membrane of a cell and the DNA is transferred into the cell. This very efficient transfer is called 'lipofection'.

Vectors as an indirect means of inserting genes into plants

Vectors are carriers of genes. We have just learned about two common vectors that are used to indirectly transfer genes to plants.

1 *Agrobacterium tumefaciens* is a bacterium that can be used to introduce genes into many different plants. It carries the gene to make the new product in its plasmid. When it infects the cells of the plant, those cells take up the plasmid and carry the genes to the chromosome in the nucleus or to the DNA in the chloroplast.

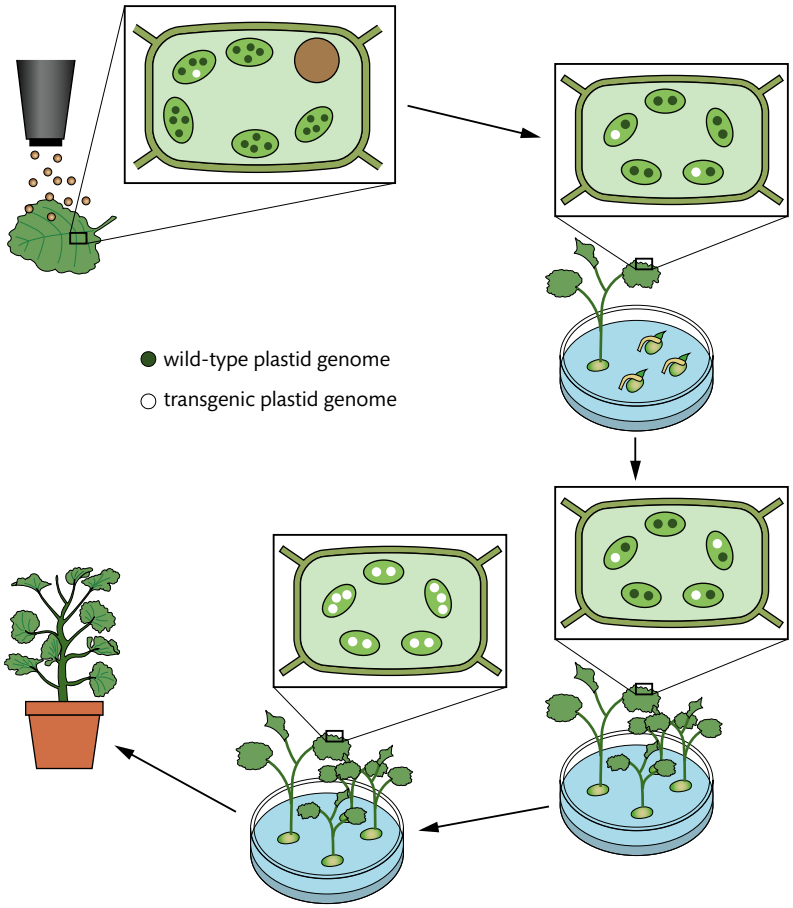


Figure 13.15 Recombinant DNA plasmids are injected into the chloroplast of the plant cell. The new DNA will be integrated into the chloroplast DNA (plastid genome).

2 Tobacco mosaic virus is a pathogen. It is used with *A. tumefaciens* to carry the genes for hepatitis antigen into tobacco plants. Bulk vaccines can then be made from the tobacco plants.

GMOs are involved in controversy in different countries regarding whether 'GMO' should be put on food labels. Are GMOs labelled in your country? Why or why not?

To learn more about bioinformatics and GenBank, go to the hotlinks site, search for the title or ISBN, and click on Chapter 13: Section B.2.

Identifying a target gene using bioinformatics

Bioinformatics combines computer science and information technology in an attempt to understand biological processes. It has been used to sequence whole genomes. You have heard of the Human Genome Project. That project has sequenced the whole human genome using information technology and computers. The genomes of many bacteria, plants, fruit flies, worms, etc., have been sequenced.

Imagine that you want to retrieve the DNA sequence of a target gene, for example the gene that makes soybeans resistant to glyphosate. Sounds tricky, doesn't it? It was very tricky before the databases used in bioinformatics were constructed. Now you can go to a database such as GenBank and find the gene you are looking for.

Using an open reading frame

The gene you are looking for in the database will be an open reading frame (ORF). An ORF is a length of DNA that has a start code of ATG and does not exhibit any of the stop codes (TAA, TAG, TGA). An ORF must have sufficient nucleotides to code for a polypeptide chain or a series of amino acids making up a protein. Usually about 300 nucleotides separate the start code from the stop code. When a scientist is looking for a protein and has the DNA sequence, he or she can go to the National Center for Biotechnology Information (NCBI), a public site, and use the ORF finder to find the protein-coding region for the target DNA sequence. This is called ORFing!

Linking the target gene to other sequences that control its expression

When a scientist is working with DNA that is to be transferred into a vector like agrobacter, the gene must undergo several modifications in order to be effective. The following diagram (Figure 13.16) is a representation of a transgene, an artificially designed construct, containing the necessary components for integration into a plasmid and production of a protein.

Figure 13.16 Representation of a transgene.

- A promoter gene must be present in order for a gene to be translated into the protein product.
- The transgene is the target gene (for example the gene for resistance to glyphosate).
- A termination sequence signals the end of the gene sequence.
- A marker gene tells the scientist if the construct has been successfully taken up by bacterial plasmids that will carry it to the plant.

CHALLENGE YOURSELF

14 In this example two of the three possible reading frames are open. Which one is not open?

(a) ...G CTC AAA ATG GGT CC....

(b) ...AA ATC TGA AGT GAT CC...

(c) ATC ATT AAT TTT TGC C...

Because it is so important to remember that ATG has to be at the beginning of an ORF, and if it is in the middle it is not an ORF, use this mnemonic to remember it: A = All, T = That, G = goes. It is always good to use mnemonic devices to remember obtuse facts!

Inserting recombinant DNA into the plant cell

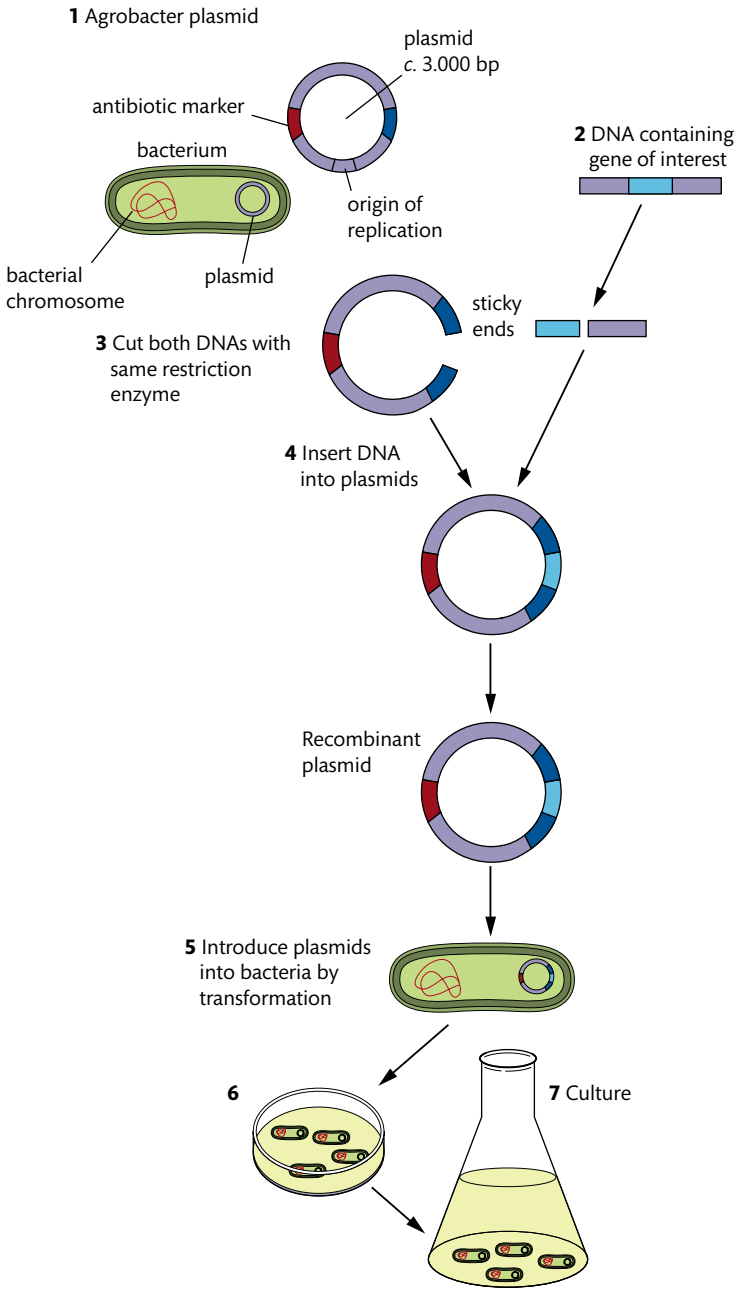


Figure 13.17 Recombinant DNA of an agrobacter plasmid and a gene of interest.

The following describes how you would put a gene of interest (in this case glyphosate resistance) into the plasmid of a vector (in this case agrobacter).

1. Engineer the plasmid DNA from the bacterium (agrobacter) by adding a marker gene. The marker gene will give antibiotic resistance that will be necessary in a later step. (Notice the bacterium has its own circular chromosome and the plasmid DNA. Only the plasmid DNA is used.)
2. Obtain the DNA of the gene of interest from another organism.
3. Cut both DNAs with the same 'molecular scissors', which are called restriction enzymes. Doing this gives them the ability to stick together and attach.
4. The sticky ends attach and the target gene is placed in the plasmid.

If the cells have been transformed by the new plasmid, they will grow on the antibiotic media. The new plasmid makes the cells resistant to the antibiotic. The gene of interest is also in the plasmid.

5 Introduce the recombinant DNA (the target gene from another organism + plasmid DNA) back into the bacteria.

6 Spread the cells on nutrient medium containing an antibiotic. Will the cells grow if they do not have the plasmid? No, they will not grow. The antibiotic will kill them. But if they have the plasmid they will be resistant to the antibiotic, so you can tell if the plasmid has been taken up by the cells.

7 Grow the cells with the plasmid in a culture vessel.

Methods of inserting recombinant DNA into plants

Recombinant DNA can be introduced into whole plants, leaf discs, or protoplasts. After inserting the DNA, the plant will be genetically modified. Genetic modification can be used to increase crop yields or produce novel products. The following are descriptions of each method.

- Leaf discs. For example, discs removed from tobacco plants are incubated with the genetically engineered agrobacter for 24 hours. Eventually the plant cells will acquire the DNA from the bacteria.
- Whole plants. Submerge the plant in a bacterial solution containing the modified plasmid. Apply a vacuum to help force the bacterial solution into the air spaces between the plant cells. Agrobacter will move the plasmid into many of the cells of the plant.
- Protoplasts. By microinjection or biolistics (see above).

Glyphosate-resistant crops (GRCs) have both risk and benefits. The benefits include a reduced need for the use of fossil fuel for tillage and a much lower use of other more toxic herbicides that affect our soil and water. However, there is a risk that GRCs might directly alter food safety. Much controversy about GRCs exists in the world community.

To find out more about GMO, GenBank, and NCBI, go to the hotlinks site, search for the title or ISBN, and click on Chapter 13: Section B.2.

Exercises

5 Describe three physical methods of introducing recombinant DNA into plants.

6 Describe glyphosate resistance in soybeans.

NATURE OF SCIENCE

Developments of scientific research follow improvements in apparatus: using tools such as the laser scanning microscope has led researchers to deeper understanding of the structure of biofilms.

B.3 Environmental protection

Understandings:

- Responses to pollution incidents can involve bioremediation combined with physical and chemical procedures.
- Microorganisms are used in bioremediation.
- Some pollutants are metabolized by microorganisms.
- Cooperative aggregates of microorganisms can form biofilms.
- Biofilms possess emergent properties.
- Microorganisms growing in a biofilm are highly resistant to antimicrobial agents.
- Microorganisms in biofilms cooperate through quorum sensing.
- Bacteriophages are used in the disinfection of water systems.

Applications and skills:

- Application: Degradation of benzene by halophilic bacteria such as *Marinobacter*.
- Application: Degradation of oil by *Pseudomonas*.
- Application: Conversion by *Pseudomonas* of methyl mercury into elemental mercury.
- Application: Use of biofilms in trickle filter beds for sewage treatment.
- Skill: Evaluation of data or media reports on environmental problems caused by biofilms.

Guidance

- *Examples of environmental problems caused by biofilms could include clogging and corrosion of pipes, transfer of microorganisms in ballast water, or contamination of surfaces in food production.*

Responses to pollution incidents

Fire boats battle blazing remnants of the Deepwater Horizon rig the day after it exploded in April 2010.

You may have read about the BP oil spill off the Gulf Coast of the USA in 2010. The oil gushed out of the Deepwater Horizon oil rig under the Gulf waters for days. The result was devastation of both the ecology and the economics of that area for months. It is still not clear what the full ramifications of the spill are to the fishing, shrimping, and crabbing industries in the area, all of which are very important to the Gulf states. Many different techniques were used as an attempt to clean up this environment.

Whether it be on the coast of Spain, Australia, or the USA, what response methods are used to clean up oil spills in the marine environment? Currently, there are three types of methods: physical, chemical, and bioremediation.

Physical methods used to clean up oceanic habitats include:

- booms, which collect the oil
- skimmers, which skim the oil off the top of the water
- adsorbent materials, which soak up the oil and are then collected and removed.

Physical methods used to clean up shore habitats include:

- pressure washing
- raking
- bulldozing.

Chemical methods used to clean up habitats include:

- dispersing agents, which act like soap and break up the large oil molecules into small droplets
- gelling agents, which are chemicals that react with oil to form solids.

Bioremediation agents are microorganisms that are added to the environment to speed up the rate at which natural biodegradation will occur. Fertilizer is added as a source of nitrogen and phosphate for the microorganisms to increase their activity.

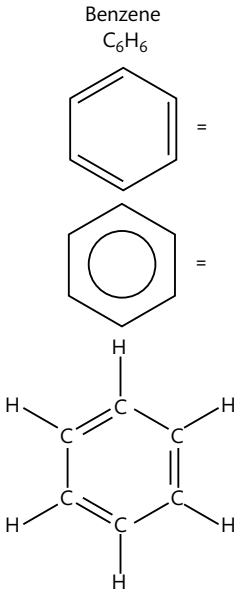
TOK

Some people think there has been a paradigm shift over the last 50 years regarding waste disposal. In the 1950s it was common to dump wastes into rivers and streams or into the soil. Sometimes people changed the oil in their car and just dumped the oil on the ground. Boaters dumped their waste in the water. Industry used lakes and rivers to get rid of their waste. If you agree that a paradigm shift has occurred, what has caused it? Explain.

Bioremediation

Bioremediation is the process of using an organism's metabolism to break down pollutants. (Check back to Section A.1 to review the different metabolic strategies of microorganisms.) The result is that environmentally undesirable properties of a substance disappear. Many microorganisms can be used to decontaminate an area, because they have the right enzymes to break down the long chains of hydrocarbon molecules that are found in organic pollutants. The products produced after the breakdown are environmentally neutral.

Figure 13.18 The structure of benzene.



Bioremediation of benzene by *Marinobacter*

An example of bioremediation of a hydrocarbon pollutant is the action of *Marinobacter* on benzene. During oil exploration, by-products of the extraction process are very salty water, called brine, and benzene. Brine is also referred to as produced water. As most microorganisms cannot live in high salt concentrations, bioremediation of the by-products of benzene can only be accomplished by a salt-tolerant species (a halophile, meaning salt-loving). Benzene is extremely undesirable in the environment because it is very stable (and so long lasting) and a known carcinogen. However, when *Marinobacter* breaks down benzene the product is simply carbon dioxide.

In an experiment using *Marinobacter*, published in the *Journal of Applied and Environmental Microbiology* in September 2003, it was shown, by using genetic analysis, that the bacteria *Marinobacter* was the dominant member of a culture mix that degraded benzene consistently over a 2.5-week period at room temperature in brine conditions (see Figure 13.19). After 4 weeks all of the products of benzene degradation had been converted to carbon dioxide.

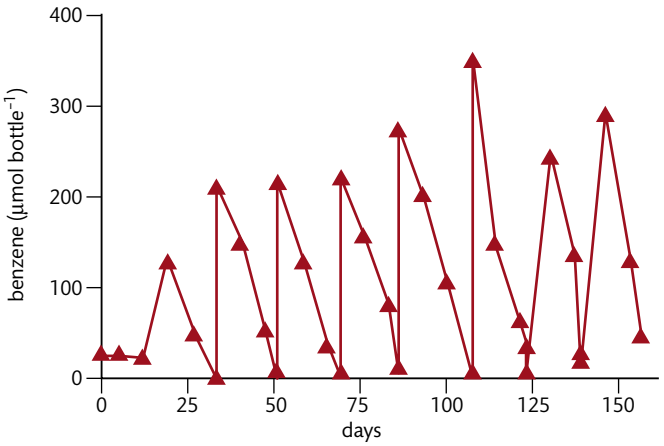


Figure 13.19 Repeated use of benzene (μ) as the sole carbon and energy source in the presence of 2.5 M NaCl by microorganisms. The cultures were maintained in 1-l capacity bottles at room temperature. After an initial lag period, the bacteria degraded 200–300 μmol of added benzene bottle^{-1} consistently in 2.5 weeks. The results for only one bottle are shown; duplicate enrichments behaved similarly. Nicholson and Fathepure 2004

Bioremediation of oil by *Pseudomonas*

Oily waste water poses a hazard for both marine and terrestrial ecosystems. Physical and chemical clean ups do not degrade the oil satisfactorily. Biodegradation is the preferred method for degrading oil, resulting in compounds that do not damage the ecosystems.

In August 2005 an article was published in the *Journal of Zhejiang University Science* demonstrating that *Pseudomonas aeruginosa* can biodegrade crude oil if another molecule is present. That other molecule is rhamnolipid, which is an effective emulsifier (surfactant) and creates much more surface area upon which the

microorganism can act. The process works even better if a second molecule is present; that molecule is glycerol. It is hypothesized that glycerol gives *Pseudomonas* extra nutrients. In the experiment published in the article, using both glycerol and rhamnolipid, 58% of the crude oil was degraded.

To learn more about using bacteria to clean up oil spills, go to the hotlinks site, search for the title or ISBN, and click on Chapter 13: Section B.3.

CHALLENGE YOURSELF

In another experiment, *Pseudomonas* was used to degrade car oil left in soil. Look at Figure 13.20. Notice that various combinations were attempted. For example, *Pseudomonas*+glycerol means that glycerol (just like the experiment above) had been added to the *Pseudomonas*.

Look at the graph and answer the following questions.

15 Compare and contrast the *Pseudomonas* and the *Pseudomonas*+glycerol treatment.

16 Based on these data, what are the two best additives that allow *Pseudomonas* to be the most effective at oil degradation?

17 What do these additives provide the bacteria with?

18 What did the surfactant do to facilitate oil degradation?

19 After the *Exon Valdez* oil spill in Alaska, scientists dumped a lot of phosphates and nitrates (inorganic fertilizer) on one of the beaches and the oil was quickly cleaned up by naturally occurring *Pseudomonas*. Can you explain why?

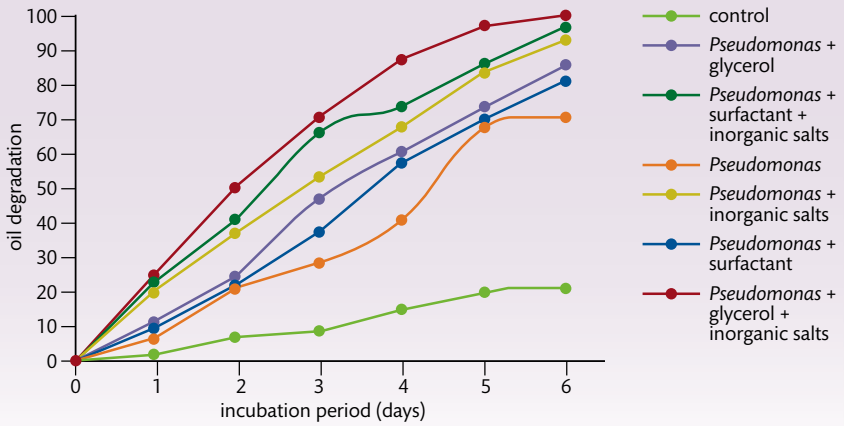


Figure 13.20 The effect of various nutrients on the degradation of car oil left in soil by the bacteria *Pseudomonas*.
Sathiya Moorthi et al. 2008, Fig. 1

Pseudomonas also cleans up mercury pollution

Mercury from substances such as discarded paint and fluorescent bulbs pollutes our environment. Mercury can leach into the soil and water from the places where mercury-containing products have been dumped. Another bacteria, *Desulfovibrio desulfuricans*, makes the mercury more dangerous. This bacterium adds a methyl group to mercury, converting it into highly toxic methyl mercury. This toxic methyl mercury attaches to plankton that is then eaten by small fish that are then eaten by larger fish. The methyl mercury builds up in the bodies of fish in a process called biological magnification. Human mercury poisoning has been attributed to ingestion of methyl mercury.

Pseudomonas comes to the rescue again. It first converts the methyl mercury to mercuric ions, and then changes the mercuric ions to the relatively harmless form of elemental mercury.

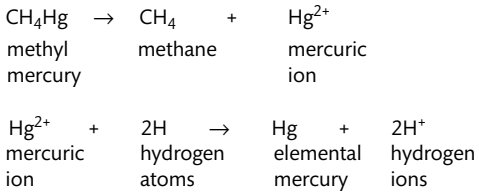


Figure 13.21 Formulas for the bioremediation of mercury.

Very few countries have sufficient resources for combating oil spills and other pollution incidents on their own. Norway therefore cooperates closely with other nations on mutual assistance in the Copenhagen Agreement. Denmark, Iceland, Finland, Sweden, and Norway are all parties in this.

Biofilms

You may have studied paradigms in your Theory of Knowledge class. A paradigm is a way thinking about a topic: it is a framework upon which to build ideas. The concept of biofilms is a new way of understanding how microorganisms exist in our environment.

Biofilms are cooperative aggregates of microorganisms that stick to surfaces like glue. We now know that biofilms affect virtually everything around us. Until recently no-one recognized that the problems we were trying to solve in industry, environment, and public health, were caused by biofilms. Biofilms cost billions of dollars a year in product contamination, damage to human health, and equipment damage. However, we have also found that they can be part of the solution to dealing with pollution in our environment, such as treating sewage, industrial waste, and contaminated soil. The research has just begun on this new paradigm of biofilms in our environment.

NATURE OF SCIENCE

Laser scanning microscopy images enable quantitative study of biofilm structure. A software suite of image-processing tools for full automation of biofilm morphology quantification has been developed. The software toolbox is implemented on a web server and a user-friendly interface has been developed to facilitate image submission, storage, and sharing. These strategies have enabled researchers to have a deeper understanding of biofilms.

Cooperative aggregates

Working in teams is always a great idea, and it seems that microorganisms have figured that out. The success of biofilms is due to the following facts.

- They are cooperative aggregates of microorganisms.
- The microorganisms can include many different types united together, such as fungi, bacteria, and algae.
- They hold themselves together by secreting extracellular polymeric substances (EPS) that stick to surfaces like glue.
- They can develop in a short time, even in hours.

Some examples of biofilms you would recognize are plaque on your teeth, which your dentist has to remove, and slimy waste that blocks kitchen drains. Even a persistent infection of a cut in your skin can be because of a biofilm.

Emergent properties

Emergent can be defined as novel and coherent structures, properties, and patterns arising during the self-organization of a complex system. In a biofilm, the properties of the biofilm community are greater than the properties of the individual components. The emergent properties of biofilms include:

- complex architecture
- quorum sensing
- resistance to antimicrobials.

Have you ever heard of desert varnish rocks? Sometimes a whole mountain range is coloured red because of the red stain of biofilms. Scraping off the stain is how petroglyphs (carvings or inscriptions on rocks) were left on cave walls. The stain is a desert biofilm.

Complex architecture

Put a clean glass slide in a pond and almost immediately a film will begin to form on the slide. The same thing happens with a tooth that has just been cleaned perfectly by the dentist. This is called a conditioning film, and it occurs in seconds as microorganisms attach to barren substrates. Videos show that certain bacteria do a little wiggle dance that helps the aggregates of cells form. As the cells join together in colonies, a more stable attachment is formed and they begin to produce EPS. Industry has invested a lot of time and money to create surfaces resistant to these attachments. It would save huge amounts of money if oil pipelines, dental drills, and medical catheters, to mention just a few items, had improved surfaces.

As many as 300 different species of bacteria can inhabit dental plaque.

TOK

Emergent properties are based on the idea that the whole is greater than the sum of its parts. Does a reductionist's view of science negate the concept of emergent properties?

Quorum sensing

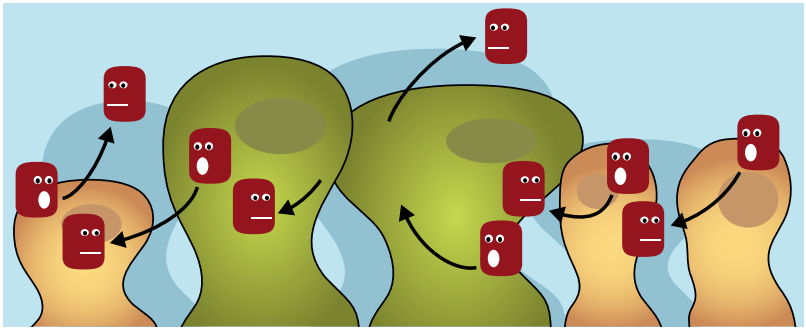
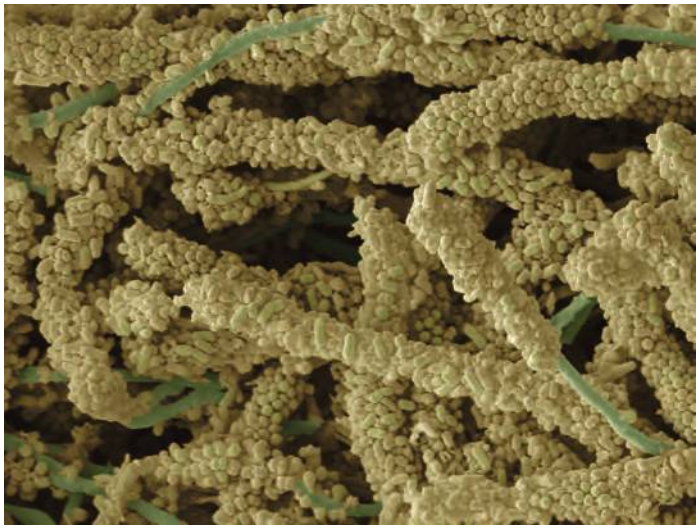


Figure 13.22 Schematic picture of cells in a biofilm 'talking' to each other in order to make more ESP.
MSU Center for biofilm Engineering, P. Dirckx



Electron micrograph of the microroganisms on your teeth forming a biofilm (plaque). Accumulation of plaque can cause dental disease in the teeth and gums due to the high concentration of metabolites produced by the biofilm.

Quorum sensing is an emergent property. Quorum sensing is the ability of microorganisms in a biofilm to cooperate with each other. Scientists have used a molecular tool called green fluorescent protein that they attach to bacterial genes to mark which genes are acting. Using the glow of the green fluorescent protein, they have discovered that when bacteria irreversibly attach to a substrate, a gene begins to make more EPS in all the bacteria. In other words, the genes make more sticky glue to adhere the bacteria even more strongly to the substrate. They seem to be able to 'talk' to each other in order to make more EPS.

Ants and honeybees use quorum sensing to make decisions about new nest sites.

NATURE OF SCIENCE

In 2001 three-dimensional X-ray crystallography was used to take the first pictures of proteins involved in quorum sensing.

As the colonies of bacteria become more dense, they can coordinate the expression of their genes in response to the density of their population. They accomplish this in the following manner.

- The first few bacteria make signalling molecules called inducers.
- Other bacteria have receptors that receive the signal of the inducer. The bacteria that received the first message then make even more inducer.
- Soon the quantity of inducer in the population is high. This stimulates the bacteria in the population to transcribe their genes all at the same time.
- A very strong biofilm of cells and matrix are made as a response of all the cells working together.

Lung infections can be caused by *Pseudomonas aeruginosa*. This bacteria uses quorum sensing to cooperate and form biofilms in the lungs. It can grow without harming the host, but when it reaches a certain population size it becomes aggressive and the biofilm becomes resistant to the immune system of the host.

Resistance to antimicrobial agents

A colour SEM *Staphylococcus aureus* biofilm found on the microscopic fibres of a wound dressing.

Biofilms are very resistant to antimicrobial agents. The fact that biofilms are implicated in human disease is of great concern to the medical community. For example, *P. aeruginosa*, which can cause infections in patients with cystic fibrosis, can exist as a biofilm. When *P. aeruginosa* has grown to a biofilm state, it is between 10 and 1000 times more resistant to antimicrobials. Biofilms can grow on implants such as hip replacements and catheters. Because of the biofilm's increased resistance to antimicrobials, the hip or catheter must be replaced, causing trauma to the patient and increased medical costs.

Research is being done to try to determine what makes biofilms resistant to antimicrobials. It may be because the polysaccharide matrix in which they live protects them, or because the biofilm is such a mix of organisms many resistant strategies have been developed. Many hypotheses have been formulated and work on this is ongoing.

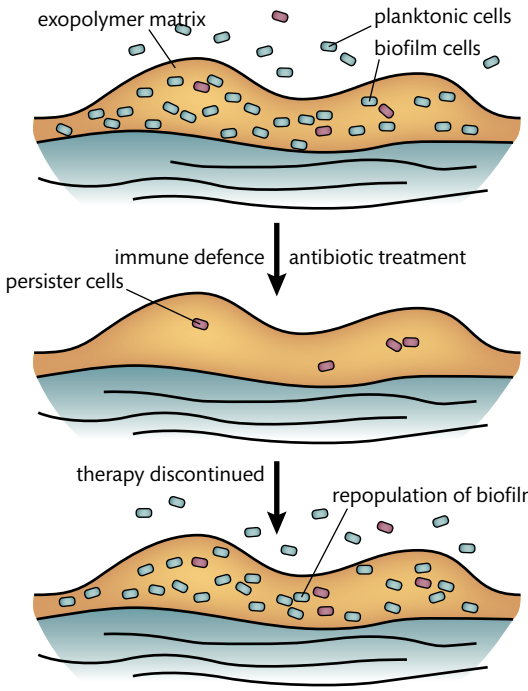


Figure 13.23 Model of biofilm resistance to antibiotics. Initially the antibiotic kills the biofilm cells (green). The immune system kills some persisters (pink). After the antibiotic treatment is reduced, the persisters repopulate the biofilm. Biofilm drug resistance: Persister cells, dormancy and infectious disease *Nature Reviews Microbiology*, 5, January, pp. 48–56, Fig. 4 (Kim Lewis 2007), Copyright 2007. Reprinted by permission from Macmillan Publishers Ltd.

Biofilms and trickle filter beds

A trickle filter is a biofilm of aerobic bacteria attached to the surface of filter media. Waste water trickles over the filter media and the attached aerobic bacteria oxidize the organic matter in the waste. The media used currently are plastic particles with high surface areas.

- The biofilm of aerobic bacteria covers each plastic particle.
- Oxygen is dissolved in the water of the filter bed and is made available to the biofilm by diffusion from the water.
- The waste water is applied with a rotary arm that causes the waste water to trickle over the media intermittently.
- The end product of this breakdown by the aerobic bacteria biofilm is carbon dioxide.
- Carbon dioxide diffuses out of the biofilm into the flowing liquid.
- Treated waste water is collected through an underwater drainage system.

This is a trickling filter system at a sewage plant in Yorkshire, England. Have you visited the waste treatment plant in your community?

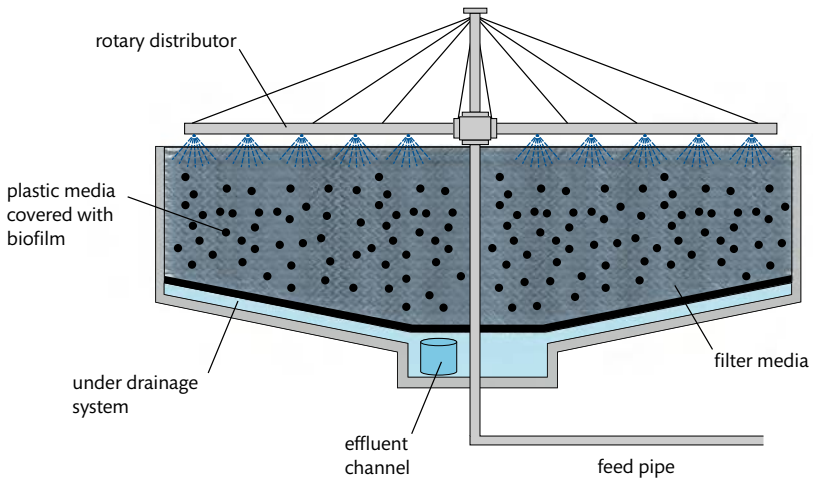


Figure 13.24 The process of trickle filtering. http://scetcivil.weebly.com/uploads/5/3/9/5/5395830/m18_l26-trickling_filter.pdf

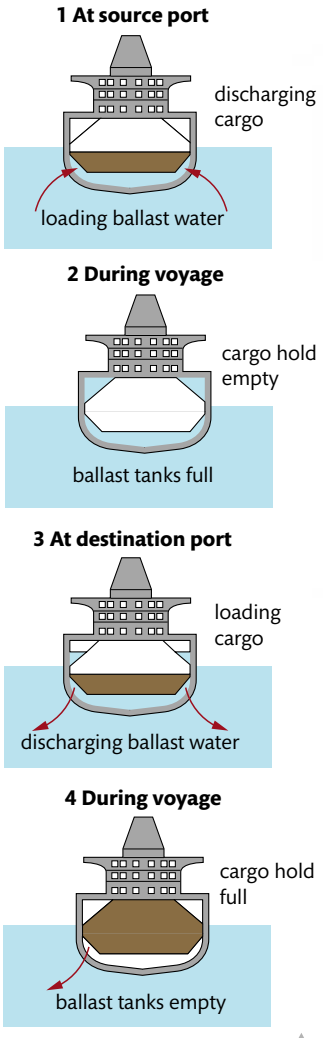


Figure 13.25 Cross-section of ships showing ballast tanks and the ballast water cycle.

CHALLENGE YOURSELF

Ship ballast water is a prominent vector of aquatic invasive species, which includes microorganisms, to coastal regions. Within a given ship, part of the microorganism population is biofilms formed on the internal surfaces of the ballast water tanks. The reasons for concern about this issue are that:

- microorganisms are much more abundant than macroorganisms (larger organisms)
- microorganisms are transferred by ship in much greater numbers than larger organisms
- once released, microorganisms, because of their small size, can easily become an invasive species
- their small size facilitates their rapid dispersal
- pathogenic bacteria, viruses, and microalga can have devastating effects on the economics of an area and the balance of the ecosystem
- microorganisms in biofilms are extremely resistant to chemical disinfectants
- field sampling has shown that 10% of ballast water tank surfaces are covered with biofilms.

A question posed by one study was as follows. Once the organisms are moved to a new location, the success of their invasion is a function of their ability to survive and reproduce. Would a temperature difference in the new water be a factor that could interfere with this ability to survive? The difference in temperature between the ballast waters and the receiving water was calculated (see Figure 13.26). Then assumptions about bacterial tolerance to temperature differences were applied.

- Tolerance: the temperature tolerance of bacteria is usually a range of 30°C. They can tolerate discharge into water that is ±15°C that of the ballast water.
- Optimality: if bacteria have an optimum range of 10°C, and if they inhabit ballast water at the midpoint of their optimal range, then their optimum growth will occur at ±5°C.

Using Figure 13.26, answer the following questions.

20 If microorganisms tolerate a water temperature ±15°C that of ballast water, then what percentage of microorganisms sampled could tolerate that new environment?

21 If the microorganisms grow at the optimum temperature, what percentage of microorganisms encountered optimal temperatures?

22 Because of the temperature differences between the ballast water and the ocean water, is temperature a limiting factor preventing the survival of bacteria discharged from ballast water? Explain.

23 Name two other environmental factors that could affect the microorganisms that are released from ballast water and that could also be studied.

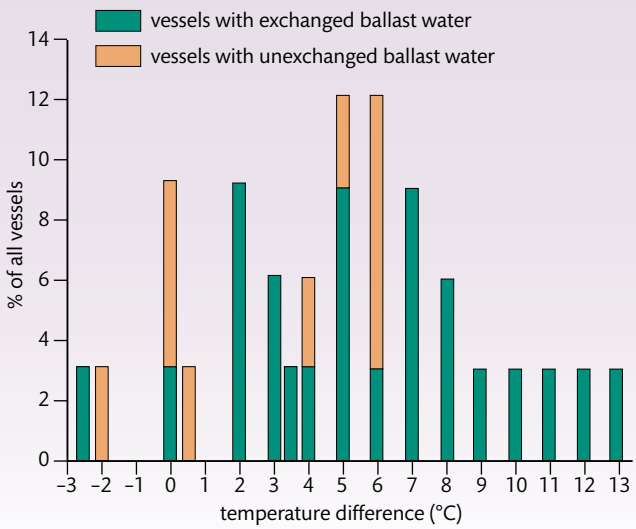


Figure 13.26 Distribution of temperature differences between undischarged ballast water and pier-side water for 32 vessels arriving at the Port of Hampton Roads. Values greater than 0 indicate the ballast water was warmer than the pier-side water. Green bars represent vessels with exchanged ballast water; yellow bars represent unexchanged water. When both exchanged and unexchanged vessels have the same temperature difference, they are stacked. The sum of all the bar values is 100. Drake et al. 2007, p. 339, Fig. 1

Biofilms may be good to use for crude oil degradation. In the second half of the 20th century oil spillage and pollution in the marine environment was a huge problem. In January 2013 researchers in India found that biofilms of *Pseudomonas* bacteria were able to degrade crude oil in a marine environment. In fact, these bacteria grew larger biomasses as they degraded the oil compared with the same bacteria living on glucose.

Bacteriophages and the disinfection of water systems

Bacteriophages are viruses found in human waste products. They are widely used for water quality assessment. Bacteriophages are organisms that are rapidly grown and easily detected. Thus they are a perfect indicator organism for the presence of human or animal waste products in water. Other viruses present in human waste are not so easy to culture and grow. Bacteriophages are helpful in assessing the resistance of viruses to the waste water disinfectant process. Studies worldwide support the value of using bacteriophages as a tool for monitoring the efficiency of waste water treatment and the disinfection process with regard to animal and human viruses.

The use of biofilm as an adsorbent of pollutant ions is one of the new technologies for treatment of contaminated water. An understanding of the properties of biofilms has allowed scientists to see their benefit in water clean-up efforts. In a study presented at a conference at Kyoto University in Japan, natural biofilms from the surface of stones were used to adsorb lithium ions and remove them from a lake.

Biofilms clean polluted waterways

Can biofilms help us clean, small polluted bodies of water? Researchers have shown that this can work. It begins with layers of mesh topped with soil and plants called rafts. Eventually, the roots of the plants will grow into the water below. Bacteria will then colonize the rafts and form sticky sheets of biofilm that coat the matrix and the roots of the plants. Biofilm bacteria use the excess nitrogen and phosphates that are polluting the waters for nutrients. They work in concert with the plant roots, which also absorb nitrogen and phosphates. The sticky biofilms also bond with other pollutants such as suspended solids, copper, lead and zinc removing them from the water.

Rafts of floating islands of plants and their biofilms can reduce the levels of pollutants in small bodies of water.

A good example of how this works can be seen in the study of Fish Fry Lake near Billings, Montana. Five years ago it was dying. As of September 2012, the algal bloom is gone, the oxygen levels are up and a community of fish has made a resurgence. This is all due to the rafts of floating island of plants and biofilm that has reduced the nitrogen concentration by 95% and the phosphate concentration by 40%. Levels of dissolved oxygen, which are so important to fish, are sixty times greater than they were at the beginning of this effort. Hopefully, new research using biofilms can helps bring back some of these polluted waterways.

Exercises

7 Briefly describe the emergent properties of biofilms.

8 List some pollutants metabolized by microorganisms.

9 Describe the use of biofilms in a trickle bed filter.

NATURE OF SCIENCE

Developments in scientific research follow improvements in technology: innovation in technology has allowed scientists to diagnose and treat disease.

B.4 Medicine

Understandings:

- Infection by a pathogen can be detected by the presence of its genetic material or by its antigens.
- Predisposition to a genetic disease can be detected through the presence of markers.
- DNA microarrays can be used to test for genetic predisposition or to diagnose the disease.
- Metabolites that indicate disease can be detected in blood or urine.
- Tracking experiments are used to gain information about the localization and interaction of a desired protein.
- Biopharming uses genetically modified animals and plants to produce proteins for therapeutic use.
- Viral vectors can be used in gene therapy.

Applications and skills:

- Application: Use of PCR to detect different strains of influenza virus.
- Application: Tracking tumour cells using transferrin linked to luminescent probes.
- Application: Biopharming of antithrombin.
- Use of viral vectors in the treatment of Severe Combined Immunodeficiency (SCID).
- Skill: Analysis of a simple microarray.
- Skill: Interpretation of the results of an ELISA diagnostic test.

Biotechnology and medicine

Biotechnology can be used in the diagnosis and treatment of disease. Biotechnology is the new medical tool of today's scientists. Improvements in technology have allowed researchers to do a better job in finding, identifying, and treating disease. Some of the diseases are genetic, but some are caused by pathogens. Pathogens are bacteria or viruses that infect us and cause an immune response. If a disease is genetic, it can be identified by genetic variations in the DNA sequence called markers.

Markers can detect a genetic disease

A marker is a genetic variation in a DNA sequence that can be observed. If a person has sickle cell anaemia, evidence for this can be observed under a microscope. We can actually see the cells becoming sickle shaped. But if someone has a predisposition to skin cancer, we can only see the genetic marker by using biotechnology techniques, some of which we will learn about in this section. These techniques have allowed scientist to be able to treat and diagnose many genetic diseases.

SNPs

If we compared your chromosomal DNA with your friend's, we would find that 99.9% of your DNA sequence is exactly the same as your friend's. In fact, if we compare your DNA with anyone else's DNA, the similarity will be the same, 99.9%. So where are the variations in that 0.1% that make us different? When scientists completed the Human Genome Project, they discovered that most human genetic variation occurs in just a very few, small, DNA sequences. Most of these genetic variations are called SNPs (snips). We can recognize SNPs when they express an abnormal protein that causes a disease, for example sickle cell anaemia. People with a normal SNP will not have sickle cell anaemia.

SNP stands for single nucleotide polymorph. This means that there is a change in one (single) nucleotide that can exist in several (poly) shapes.

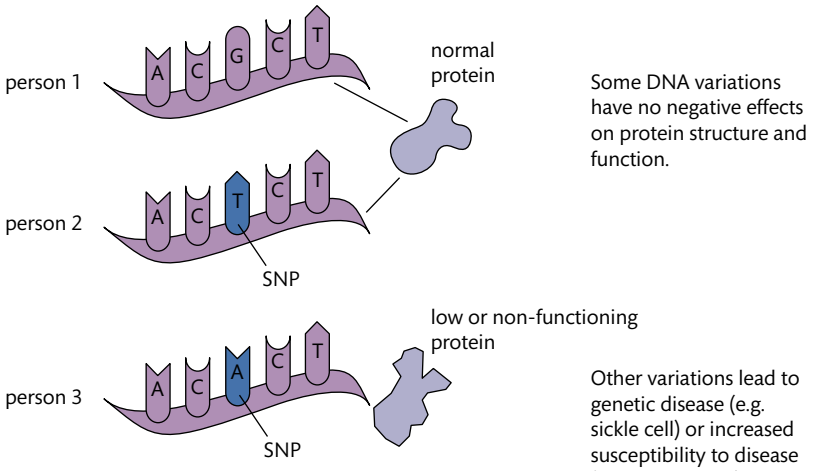


Figure 13.27 Single nucleotide polymorphisms. Thieman and Palladino 2013, p. 15

In Figure 13.27, you can see SNPs from three different people. Person 1 has a gene that expresses a normal protein. Person 2 has a T (thymine) nucleotide instead of a G (guanine) in the SNP, but also expresses a normal protein. Person 3, however, has a variation that makes an abnormal protein. This absence of a normal protein may result in disease.

TOK

The trick to solving the puzzle of disease is to understand which piece of the puzzle plays the greater role in the disease under consideration. Is it genetics or environment?

Scientists have constructed maps of SNPs. These maps point out the location of each SNP along the length of every human chromosome. This was an international project and the information is now available free worldwide.

Markers can detect predisposition to genetic disease

The presence of a genetic marker can tell us whether we have a predisposition to a certain disease. A genetic marker may be a short DNA sequence like a SNP or a longer DNA sequence. A marker indicates that we have susceptibility, but it does not mean we will definitely develop the disease. We cannot change our genes but, in some cases, we can alter our environment to prevent or delay the onset of the disease.

An example of an interaction between genes and the environment is seen in the higher susceptibility of fair-skinned people to skin cancer. Skin cancer in fair-skinned people is associated with a genetic marker. The marker tells us that there is a mutation in the melanocortin 1 receptor (MC1R) gene. If fair-skinned people are informed that they have this marker, they can take precautions to limit their exposure to direct sunlight. This may reduce the likelihood of skin cancer occurring.

NATURE OF SCIENCE

2001 was a landmark in the biotechnology timeline. At a press conference, the world's best-known molecular biologists announced the draft of the Human Genome Project. The Human Genome Project was completed in 2003 and has identified the chromosomal location and sequencing of all of the genes in the human genome. This has greatly increased our knowledge of human genetics and how to diagnose and treat human diseases.

DNA microarrays

A DNA microarray is a collection of DNA probes attached to a solid surface which can be used to identify a genetic marker. A small amount of blood or other source of DNA is collected and attached to the microarray. A DNA microarray is also called a gene chip. The gene chip is spotted (marked) in precise locations with single strands of thousands of short, single-stranded, known DNA in a grid-like pattern. Each spot has multiple copies of a known gene. This technology allows scientists to see the expression of genes by looking at the messenger (m)RNA that is transcribed by the gene.

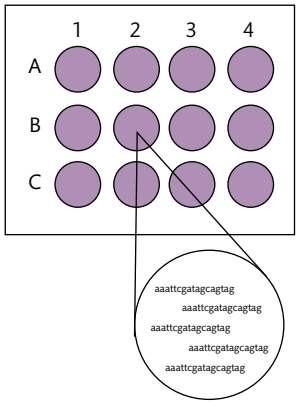
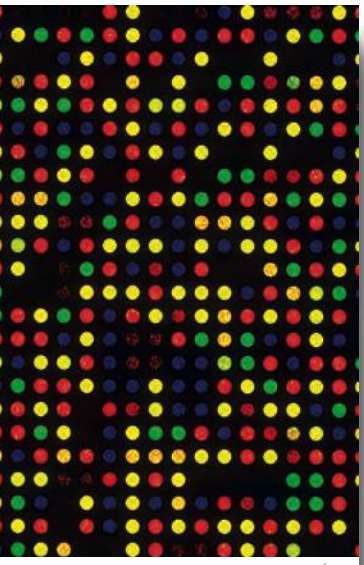


Figure 13.28 A spot grid. http://www.urmc.rochester.edu/MediaLibraries/URMCMedia/life-sciences-learning-center/documents/DNA_Microarrays_and_Cancer.pdf, p. 13



A DNA microarray.

Detail of DNA microarray

Look at the Figure 13.29 and the sequence described below.

1 From a blood or tissue sample, mRNA is isolated. Remember that mRNA is the molecule that takes the message from the DNA.

2 From the mRNA a single strand of copied DNA (cDNA) is made using an enzyme called reverse transcriptase (RT). The new cDNA will be a copy of what was originally in the blood or tissue sample but it is only a single rather than a double strand. The cDNA will also be made of molecules (nucleotides) that have fluorescent dyes attached to them as labels.

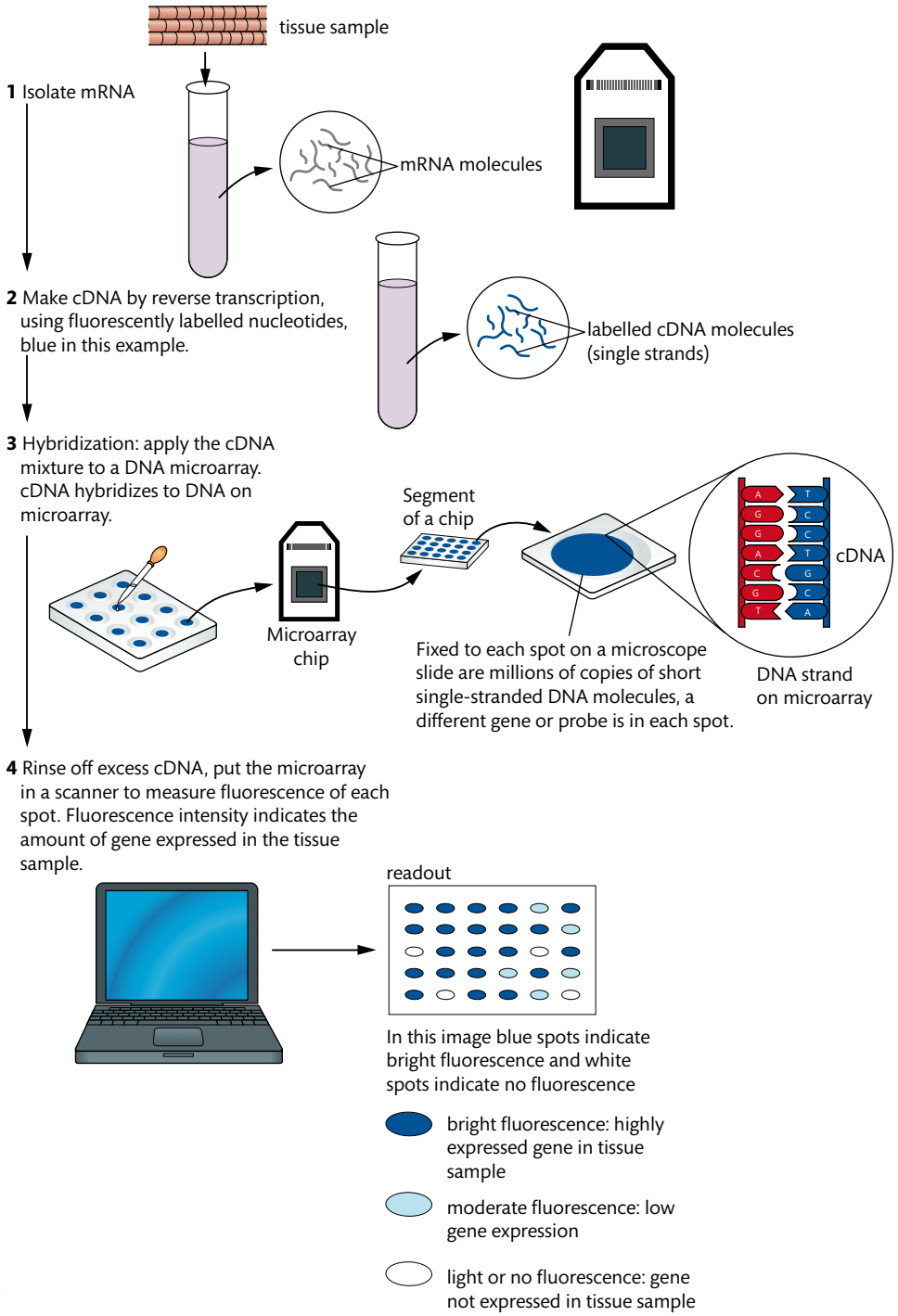


Figure 13.29 A DNA microarray. Thieman and Palladino 2013, p. 87

3 Hybridization: in the microarray there are many probes attached representing thousands of regions of DNA. The probe is a short section of DNA that will pair (hybridize) with cDNA from the blood sample. Because one microarray contains many probes, it can identify many genetic markers at the same time.

4 Excess cDNA that did not hybridize with a probe is rinsed off. The darker the colour, the more cDNA has attached to a probe. Because we know what is in each probe, if the cDNA hybridizes with a probe we now know what DNA was present in the original sample. The intensity of the fluorescence of each probe is measured.

The process of using a DNA microarray is:

- **isolate mRNA from a sample**
- **translate mRNA into cDNA (single-stranded DNA)**
- **label the cDNA with florescent labels**
- **hybridize the cDNA in question with known DNA on the probes of the microarray**
- **rinse off excess cDNA from the microarray**
- **complementary cDNA will bind to the probe**
- **analyse the colours present in the microarray.**

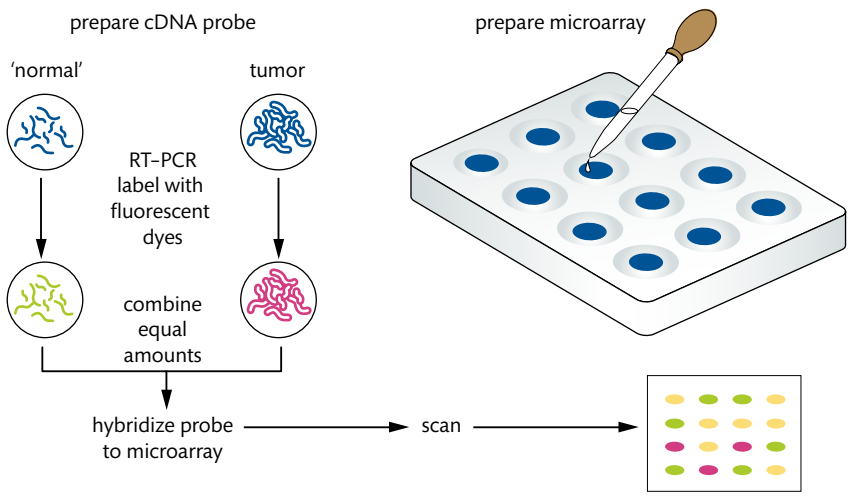


Figure 13.30 Microarray technology.
http://www.genome.gov/Pages/Hyperion/DIR/VIP/Glossary/Illustration/Images/microarray_technology.gif
Courtesy: National Human Genome Research Institute

Notice in Figure 13.30 how the cDNA matches up with the DNA on the microarray. Bright fluorescence means the gene in the probe has matched with the gene in the blood or tissue sample.

This is a gene chip of all the genes of a frog. All human genes are also available on a gene chip.

Analysis of a simple microarray

Use the hotlinks at the end of this section to see a complete tutorial of this analysis. This example is adapted from the website.

Assume you are an oncologist (a doctor who treats cancer) and your patient has skin cancer. You want to determine exactly how the cells in the patient's normal skin differ from the skin cancer cells. The method you use includes a gene chip that contains the DNA probes for the microarray. The method is described below.

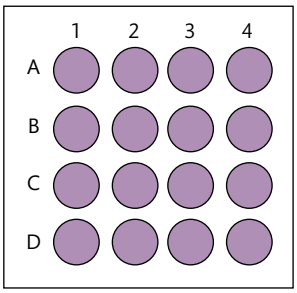


Figure 13.31 A microarray plate with human genes attached.

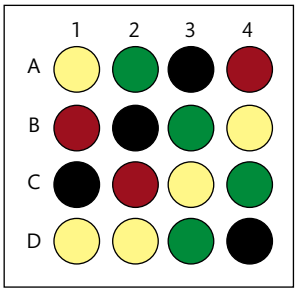


Figure 13.32 cDNA tagged with red and green dyes have attached to probes on the microarray plate.

- Take a sample of cells from a cancerous area of the skin and another sample from normal skin (these are the tissue samples).
- Extract mRNA from the cancer (cancer mRNA) and mRNA from the normal (normal mRNA) cells.
- Add the enzyme RT to each mRNA. RT is reverse transcriptase that will allow mRNA to make cDNA (which is much more stable than mRNA).
- In a tube with the normal mRNA, add DNA nucleotides tagged with green fluorescent protein. This makes this cDNA green.
- In a tube with the cancer mRNA add nucleotides tagged with a red fluorescent colour. This makes this cDNA red.
- Use a microarray plate (microchip) purchased from a biotech company that has all the human genes attached to the plate. Each square has multiple copies of a single gene. A computer keeps track of where each human gene is located on the plate.
- Pipette the red and green cDNA molecules onto the plate. Allow attachments to form and rinse off cDNA that does not hybridize with a probe on the microarray.
- The results are a grid of coloured spots (Figure 13.32) that can be interpreted.

A green spot indicates that the gene is not expressed or turned on in the cancer cell. It is only expressed in the normal cell. This gene may be involved in prevention of skin cancer.

A red spot indicates that the gene is expressed or turned on in the cancer cells but not in normal cells. This gene may be involved in causing skin cancer.

A yellow spot indicates that the gene is expressed or turned on in both normal and cancer cells, thus there is no difference here between the cancer cells and the normal cells. This gene is probably not involved in causing skin cancer.

A black spot indicates that the gene was not expressed in either type of cells. Because there is no difference this gene is probably not involved in causing skin cancer.

CHALLENGE YOURSELF

Use Figure 13.32 to review what you have learned about analysis of microarrays.

24 How many genes in this microarray are turned on in the cancer cell?

25 Name the genes.

26 How many genes in this microarray are turned off in the cancer cell?

27 Name the genes.

28 What does yellow indicate?

29 Name the genes.

30 What does black indicate?

31 Name the genes.

32 Which genes could be the genes causing cancer? What is your evidence?

33 Which genes could be preventing cancer? What is your evidence?

34 If an mRNA sequence is AAU AGG UAC ACG, what is the sequence on the DNA microarray for this?

Using genetic material or antigens to detect infection by a pathogen

Microarrays can be used to detect and identify a pathogen. For example, a company called Affymetrics has developed the SARSCoV GeneChip, which contains 30 000 probes for the entire genome of the SARS virus. SARS virus is a very contagious respiratory virus that has infected thousands of people since its discovery in 2002. Using this gene chip, the genetic materials of the virus can be identified. This helps track the pathogen and the outbreaks of illness it causes.

In 2009 swine flu, or influenza A, caused a global pandemic. It had rapid human-to-human transmission and unknown virulence. With this type of outbreak, fast and sensitive detection is required for diagnosis. An antigen microarray against this influenza was developed. This was able to detect the presence of antigens causing the influenza.

PCR is used to detect strains of flu virus

How do scientists take a minute dot of blood from a crime scene and amplify it so that it can be used as evidence? In the same way that they can amplify DNA from a fossilized dinosaur: they use a technique called PCR. PCR stands for polymerase chain reaction. PCR is a procedure that takes short segments of DNA and amplifies them so that they can be identified. Untold numbers of copies of DNA can be made by PCR (amplification). This is another technical improvement that has allowed scientists to diagnose and treat disease.

Healthcare professionals need fast and accurate tests on hand to distinguish one type of influenza (flu) from another. PCR is such a test. Epidemiologists need accurate data to predict the spread of the flu from one country to the next. PCR results can provide that data.

PCR can be used to test nasal secretions. Currently, it is the most sensitive test for the flu virus. It can diagnose influenza A and B and H1N1 flu viruses.

The following is a description of the PCR process.

1. Starting materials: target DNA collected from nasal secretions; nucleotides to use for making copies of DNA; DNA polymerase primer that gets the new copy started.
2. Denaturation: the DNA is heated to cause it to separate into two strands.
3. Hybridization: the DNA is cooled slightly to let the primers hybridize with the DNA at the 3′ end.
4. Extension: DNA polymerase adds new nucleotides to the 3′ end of each primer to build complementary strands.

At the end of each cycle the amount of target DNA has been doubled. After every 20 cycles of PCR, approximately 1 million copies of target DNA have been produced.

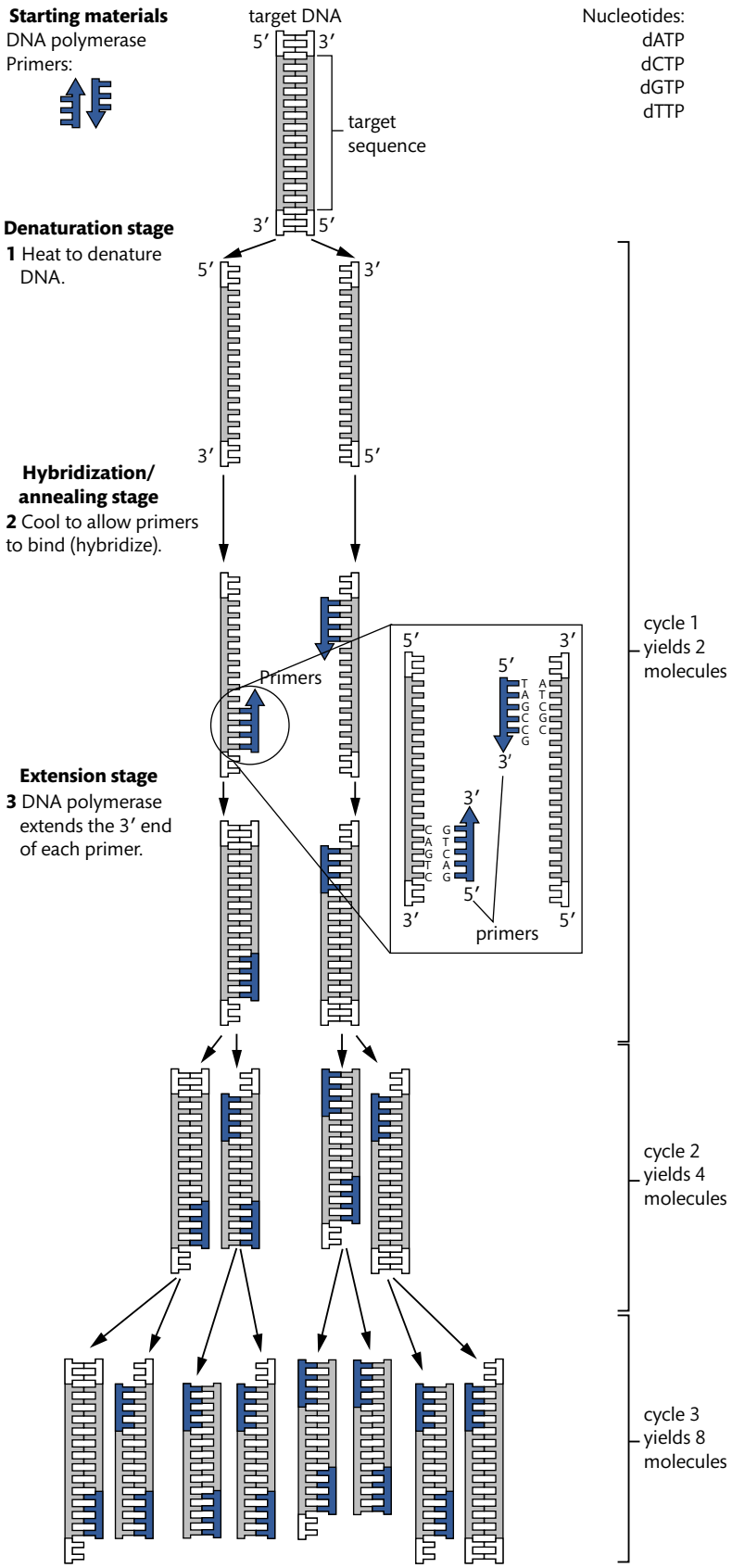


Figure 13.33 The polymerase chain reaction (PCR). Thieman and Palladino 2013, p. 73

Metabolites that indicate disease

A doctor may draw blood and collect a urine sample as part of a physical examination. The doctor is looking for metabolites that are biomarkers that indicate disease. What can be determined by examining these body fluids has been greatly improved by biotechnology. Now laboratories perform tests such as the microarray and PCR, and many others, to look for metabolites that indicate disease. Examples of some biomarkers are as follows.

- PSA for prostate cancer: tests for this biomarker look for elevated levels of prostate-specific antigen (PSA). If the PSA is elevated, it indicates possible prostate cancer.
- S100 for melanoma: this is a protein biomarker that if elevated indicates a high number of cancerous melanoma cells. Treatment of the melanoma should lower the protein biomarker levels.
- HER2 for breast cancer: 20–30% of breast cancer patients have higher than normal expression of this biomarker. It is important to monitor the level during treatment for some patients.

These are just a few examples of biomarkers that have been discovered; much research is ongoing in this field. For example, scientists are on the verge of finding a biomarker to predict the onset of Alzheimer's. This predictive metabolite might allow patients to slow down the onset of the disease. Again, innovations in technology have allowed scientists to diagnose and treat disease.

One type of biomarker is a tumour marker. Tumour markers can be used to help diagnose cancer or to check a patient's response to treatment. Tumour markers are proteins that show a change in gene expression. In 2013, 20 different tumour markers were in clinical use. For example, Marker CA15-31CA27.29 for breast cancer is found in the blood. It is a tumour marker that can be used to assess whether a treatment is working for a patient or if the breast cancer is recurring.

ELISA diagnostic test

The enzyme-linked immunosorbent assay (ELISA) is a diagnostic tool that was the first test widely used for the screening of human immunodeficiency virus (HIV). It can determine whether there is any HIV antibody in the blood. ELISAs are also used to test for the presence of drugs in blood and urine.

An ELISA diagnostic test can be used to screen blood donors.

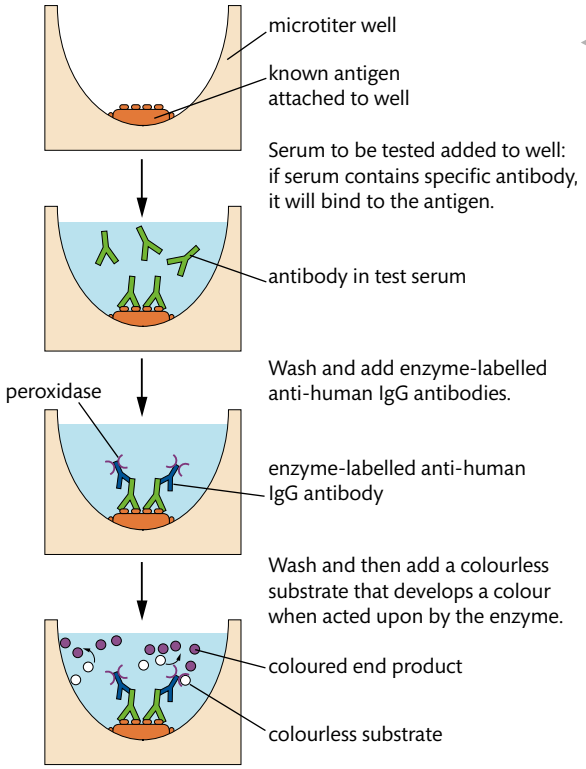
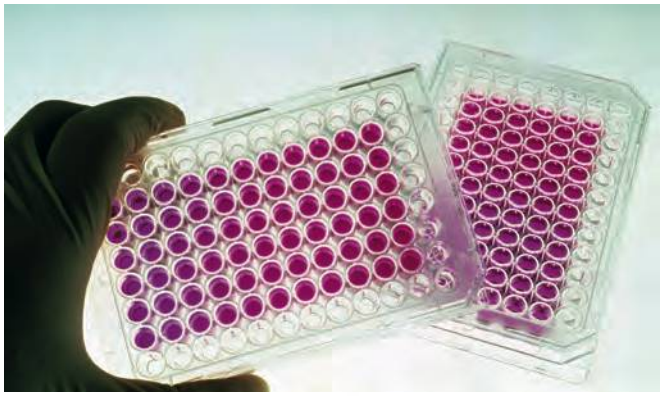


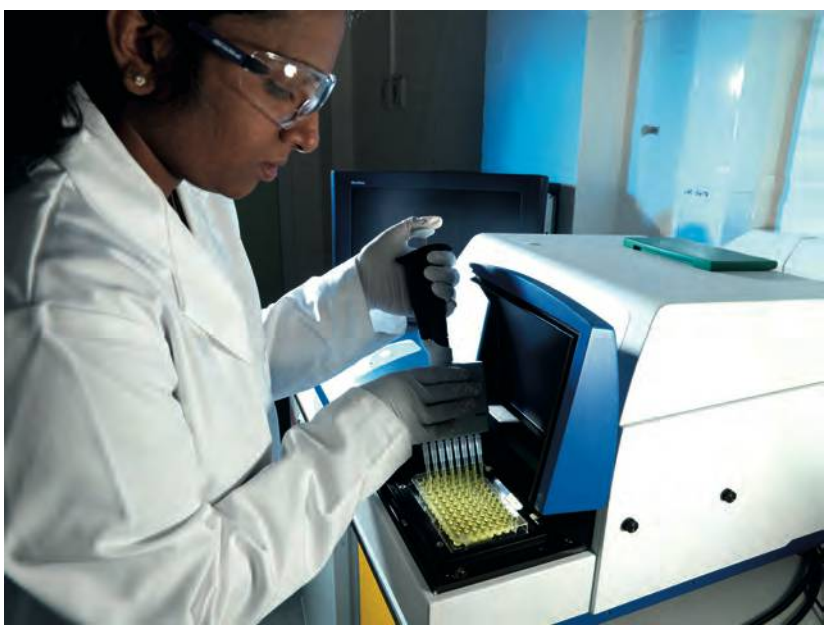
Figure 13.34 Enzyme-linked immunosorbent assay (ELISA). Nester et al. 2008, p. 445



Microtitre plates.

The steps for doing a direct ELISA test are as follows. (Follow Figure 13.34 as you read these steps.)

- Take a blood sample.
- Centrifuge to separate the cells from the blood serum.
- Decant the serum; it is the serum that will contain the antibodies.
- Use a microtitre plate with a known antigen (gold) attached to each well.
- The serum to be tested is added to the wells. The antibodies (green) that are specific to the attached antigen will bind to it.
- Wash off the plate to remove anything that has not attached.
- Add another molecule, an enzyme-labelled antihuman IgG antibody (blue), to create colour. This antihuman IgG antibody (blue) has an enzyme attached (peroxidase which is purple) that will change colour when a colourless substrate is added.
- Wash again to remove all unbound antibodies.
- Add a colourless substrate. The enzyme will act on it to produce a coloured end product. The stronger the colour, the higher the original quantity of antibody that was present in the serum.
- The colour is quantified in a plate spectrometer reader. The spectrophotometer is set at an appropriate nanometre setting in order to give the best reading of the optical density of the colour change.



A plate reader.

Numbers representing the optical density of the colour change being measured are reported in ELISA tests. For example, if the ELISA test is used for drug screening in the workplace, there is a relevant cut-off number for positive and negative tests. Above a certain number would be considered a positive drug test.

The ELISA test is typically used to test donated blood for the presence of HIV in order to ensure the safety of the blood supply. The presence of HIV antibodies suggests evidence that the virus is present. However, the test is not perfect and results include a small number of false positives.

Since 1985 all blood donations have been screened for HIV with an ELISA test. Diagnostics are combined with careful donor screening.

Interpretation of ELISA

Look at the ELISA data from three patients tested for HIV.

Table 13.3 ELISA data for an HIV text

Positive control	Negative control	Patient A	Patient B	Patient C	Assay control
1.869	0.143	0.045	0.312	1.989	0.132

The numbers in the chart are the optical densities recorded by the spectrophotometer at 450 nm. Above 0.400 is a positive result for this test. Optical densities of 0.200–0.399 need to be retested. Values below 0.200 are negative. If a patient is positive, he or she will be retested using a different test to obtain more evidence of a positive result.

From the ELISA test results shown in Table 13.3, could any of the patients be positive for HIV? Answer: patient C. The optical density of patient C is over 0.400: it is close in value to the positive control. The results can be interpreted as follows.

- The colour change is very strong, indicating that the patient has antibodies to HIV that have attached to the antigen for HIV in the microtitre plate.
- The positive control is a sample known to contain the HIV antibody.
- A positive result from the positive control will tell you that the procedure is working well.
- The negative control is a sample that is known not to contain the antibody being tested.
- A negative control is a check for false positives.
- The assay control has no serum, but all the other steps are the same.

CHALLENGE YOURSELF

Review the example above. When you are confident that you understand how to analyse the data from an ELISA test, try this example without referring to the explanation above.

Here is another set of data: ELISA tests for elevated blood levels of antibodies produced in response to *Borrelia burgdorferi*, the bacteria that causes Lyme disease. If the test is performed at least 4 weeks after a tick bite, the test will identify the presence of Lyme disease.

Table 13.4 ELISA data for a Lyme disease test

Positive control	Negative control	Patient A	Patient B	Patient C	Assay control
1.765	0.189	1.535	1.892	0.435	0.202

The above ELISA data are from three patients. The cut-off value indicating a positive result is 0.500. Values of 0.300 and 0.499 are indeterminate and need to be retested. Values below 0.300 are considered to be negative. The spectrophotometer is set at 400 nm for this test.

35 Which of the patients are testing positive for Lyme disease?

36 How do you know?

37 What do the numbers measure? Explain in detail.

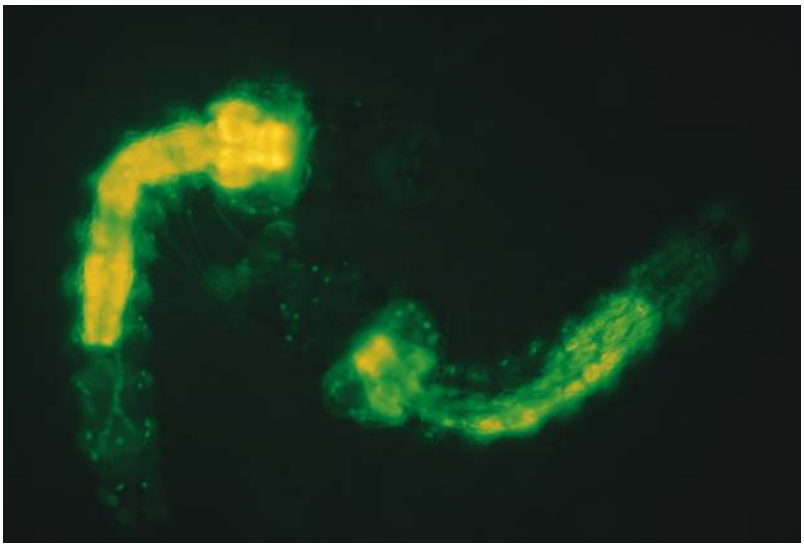
38 Does any patient need to be retested? Explain.

39 What is a positive control?

40 What is a negative control?

Lyme disease is named after a town in Connecticut where the first cases were discovered in 1975. In 1981, Willy Burgdorfer identified the bacteria that causes the disease.

Tracking experiments

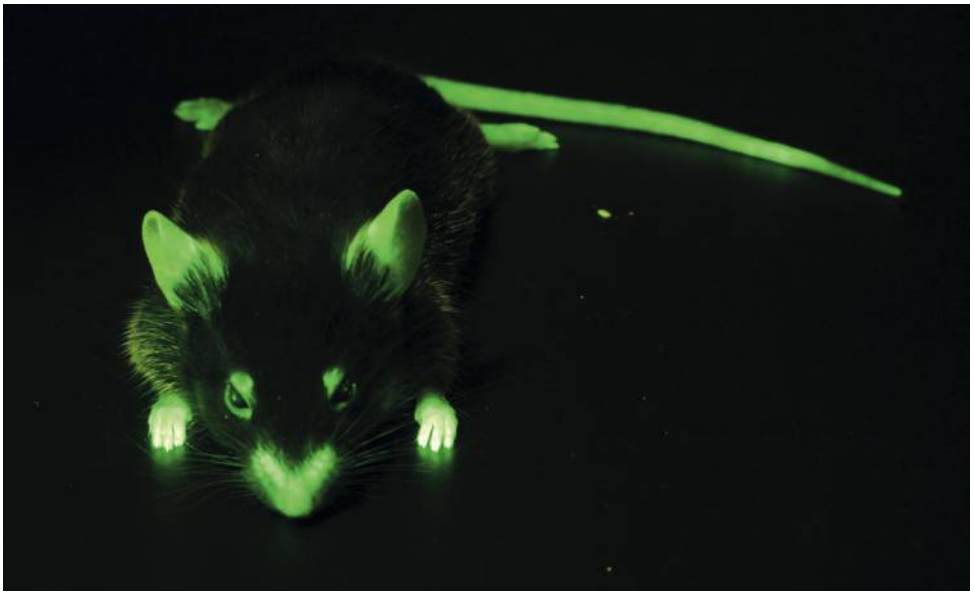


Mosquito larva.

Tracking experiments are used to gain information about the localization and interaction of a desired product. A method of tracking is to use a reporting element such as green fluorescent protein (GFP) so that the product can be visualized.

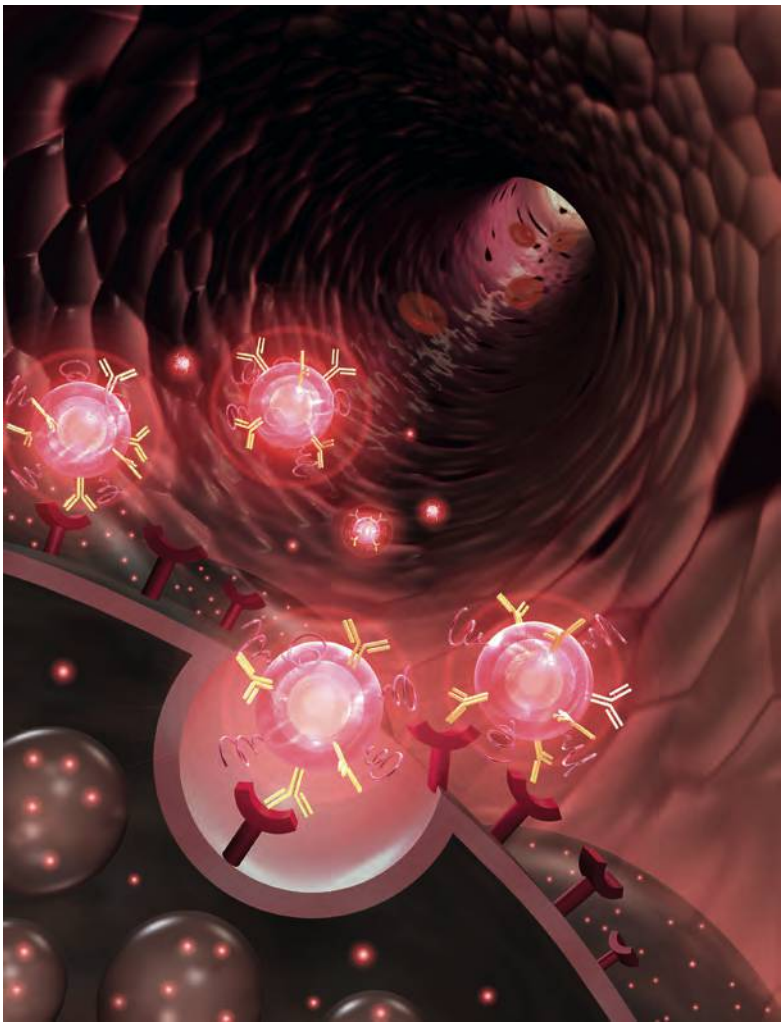
- GFP can be spliced into the genome of an organism in the region that codes for a target protein.
- In the cells where the gene is expressed (the protein is produced) the GFP is also produced.
- Thus only the cells expressing GFP will fluoresce and can be found using fluorescent microscopy.

GFP is a powerful tool that has been used for studying gene expression since its discovery in the 1960s, when the gene for making it was extracted from jellyfish. One example that you may already be familiar with is the use of GFP for tracking the production of insulin. Researchers can attach GFP to the cells producing insulin. This allows scientists to gain information about where the insulin is produced (localization) and its interaction with other molecules. GFP is another innovation in technology that has allowed scientists to diagnose and treat disease more easily.



A mouse expressing GFP under ultraviolet (UV) light.

Tracking with luminescent probes



Quantum dots.

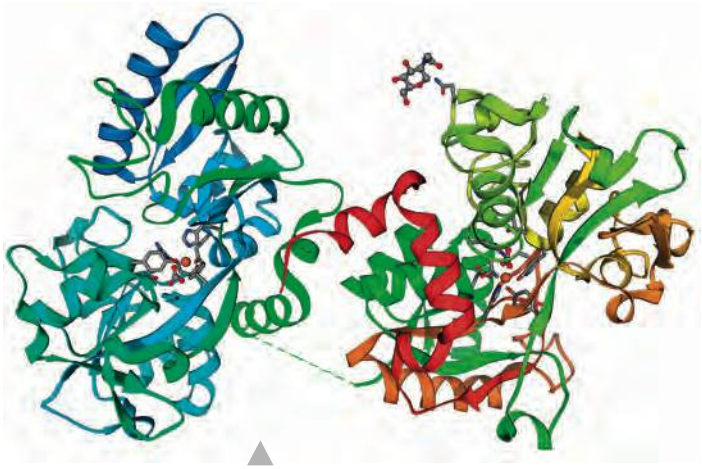
Brightly florescent quantum dots (QDs) are becoming important tools for tracking molecules in living systems. We will see how this is another tool in the toolbox of biotechnologists to enable efficient diagnosis and treatment of disease.

What are QDs? QDs are nanoparticles. They are made of semiconductor materials, such as silicon. The great benefit they have, besides being small, is that they glow a particular colour when illuminated with low-intensity light. You can see how this makes them excellent molecules to use for tracking.

The prefix nano- means 10^{-9}. QDs are nanoparticles that are 10 nm in diameter; 3 million QDs could be lined up end to end and still fit the width of your thumb.

QD drug delivery

Using the QD tracking strategy, researchers have a better understanding of how nanoparticles could deliver drugs specifically to tumours. At the Tohoku University in Japan, Dr Higuchi and his colleagues have used QDs labelled with antibodies and a fluorescent-sensitive video camera to film QDs as they travel through the bloodstream of a mouse. The dots are labelled with the antibody HER2, which seeks out and binds to a protein found on the surface of some breast cancer cells. The researchers can see how the dots travel from the injection site to the cell nucleus of the tumour cells. The goal is to attach a drug against the tumour so that it will also be carried by the QDs.



The protein transferrin can help find cancer cells. Transferrin binds to the transferrin receptor that is overexpressed in many types of cancer cells.

- **Transferrin is attached to a luminescent quantum rod.**
- **Labelled transferrin travels to the transferrin receptors.**
- **Receptors are overexpressed (for some reason the cells are making lots of receptors) on cancer cells.**
- **The labelled transferrin attaches to the cancer cells.**
- **Special microscopes can find the luminescent quantum rods and thus find the cancer cells.**
- **Anticancer drugs could be attached to the transferrin molecule.**
- **Anticancer drugs could then be delivered to a specific target cell.**

The World Health Organization estimates that 3 million people die each year from diseases that could be prevented by vaccines.

Tracking with transferrin and a luminescent probe

In another experiment, Dr Prasad at the State University in Buffalo, New York, has used quantum rods. Quantum rods can fluoresce under a wider range of colours than QDs, and can be used for imaging cancer cells. Dr Prasad and colleagues attached a protein called transferrin to the quantum rod. The quantum rod travels through the blood stream to seek out and bind to cancer cells. Transferrin binds to the transferrin receptor that is overexpressed in many types of cancer cells. Transferrin binds to the transferring receptor like a key to a lock.

Results measuring the luminescence of the quantum rods showed that only target cells took up the rods, and only target cells accumulated the rods. Other cells did not show luminescence. Transferrin only locates the cancer cells.

Research is ongoing into the use of transferrin as a delivery vehicle for anticancer drugs. The drugs could be attached to the transferrin molecule and delivered to the cells as transferrin attaches to the cell membrane.

Biopharming

Biopharming uses genetically modified plants and animals to produce proteins for therapeutic use. A variety of innovative technologies are now available that allow us to use pharmaceuticals derived from genetically engineered plants and animals to treat disease.

Biopharming using plants

Will you get your next vaccine from a potato rather than from an injection? That is the hope of many researchers. The plan is for farmers to grow medicines as well as crops. However, the ethical consideration of growing GM crops has plagued the development of these biopharmaceuticals from plants. Because of environmental concerns, many of these GMO have not reached the market yet.

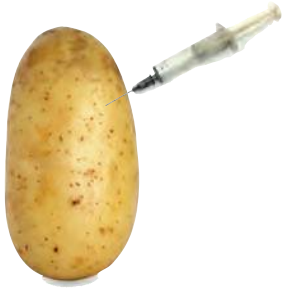
If edible vaccines could be produced successfully by genetic engineering techniques, they would produce antigens. When the antigens entered your bloodstream, they would cause antibodies to be produced that would give you immunity. The genetic engineering techniques used are the same as those we studied in Section 12.2, using *Agrobacterium tumefaciens* or tobacco mosaic virus as a vector to carry the new gene into the plant.

TOK

In 2011, a company in Wisconsin had a plant-derived hepatitis B vaccine that was about to enter the second stage of human clinical trials. The vaccine was produced by a genetically engineered potato. These special potatoes were grown indoors to prevent them from being mixed with other naturally grown potatoes in the wild. According to the company, if you need to be vaccinated, you can eat a certain amount of the potato and you would build up the antigen and be vaccinated. However, the plan is to extract the antigen and put it into a pill for ease of use. Will plants be the new pharmaceutical producers? What are the risks and benefits of this new technology? How can the most ethical decision be made?

Biopharming using animals

For decades, genetically engineered bacteria have produced simple proteins such as insulin and the human growth hormone. However, bacteria cannot make complex proteins. Bacteria are not able to produce the complex folding structures required. Only mammalian cells are capable of making these complex molecules. Animals such as goats are now making pharmaceutical proteins for us along with their milk. You may wonder why transgenic (cloned) animals are so often goats. Goats are cheaper to rear than cattle, reproduce more quickly, and produce more abundant milk. The goat that is being used to produce pharmaceuticals is cloned to have the desired gene. The cloned goat produces milk rich in the desired protein.



Vaccines made in plants could offer vital disease protection.

Biopharming of antithrombin

Transgenically derived therapeutic proteins are necessary for genetic disorders such as haemophilia. Haemophiliacs lack a functional clotting protein called antithrombin III. Transgenic goats now produce this protein. Using transgenic animals to produce protein such as this is cost effective, has a guaranteed production capability, and offers a safer, pathogen-free product.

Transgenic goats can now produce a clotting protein needed by haemophiliacs.

Gene therapy uses viral vectors

Viral vectors are a tool commonly used by molecular biologists to deliver new genetic material into cells. Imagine if you had a genetic disease for which there was no cure. You then found out that a new technology using a viral vector had been developed that would supplement your defective gene with a normal gene. It involved using a virus to infect some of your cells, but the virus was the carrier of the gene you needed. When the virus infects your cells, it brings the normal gene with it. This might make you apprehensive but also gives you hope of a cure.

This technique is called gene therapy. Biotechnologists are constantly working to improve how this system works for patients. Two of the recent successes are described briefly below.

- In 2011, haemophilia B, caused by the absence of a coagulation factor, was successfully treated. A virus called AAV8 delivered the missing gene. So far six patients have begun to produce the factor again.
- MLD (metachromatic leukodystrophy) is a disease that is fatal in early childhood. It is cause by a defective gene called ARSA. The defective gene causes brain and spinal cord degeneration. Viruses were given a working copy of the gene and injected into three young patients' bone marrow cells. The defect was corrected and new blood cells were made containing the new gene. The result was that all three children then had high amounts of the necessary ARSA and their central nervous systems stabilized.

NATURE OF SCIENCE

Scientific journals are news magazines with articles on current research in specific fields of research. The MLD success was reported recently in the journal *Science*. What other success stories or failures about gene therapy have been reported in scientific journals?

Steps in gene therapy

Most gene delivery strategies rely on viral vectors to introduce therapeutic genes into cells. For a viral vector to work, it has to be genetically engineered so that it will not produce disease or spread throughout the body and infect other tissues. The following steps outline the method of gene therapy.

- Genetically disable the virus so that it cannot affect other tissues.
- Clone the normal gene to be given to the patient.
- Incorporate the cloned gene into the virus that will deliver the gene
- Remove cells from the patient that contain the defective gene.
- Culture the cells with the virus so that it will infect the cells and deliver the normal gene to the genome of the patient.
- Reintroduce the genetically altered cells back to the patient.

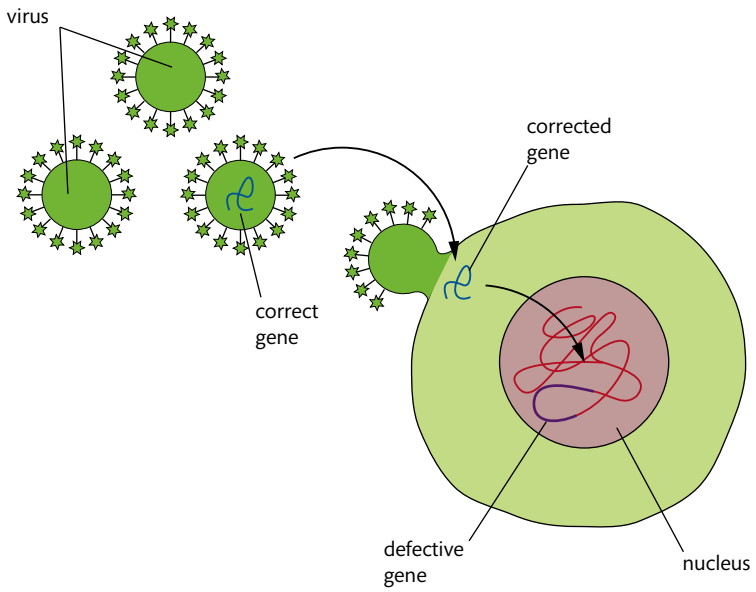


Figure 13.35 Gene therapy. Seen here is technique for treating disease by altering a patient's genetic material. Gene therapy works by introducing a normal copy of a defective gene into the patient's cells. http://www.genome.gov/glossary/index.cfm?p=viewimage&id=77 Courtesy: National Human Genome Research Institute

To help you remember a list of procedures like the one for SCID, shorten the steps and then make a mnemonic device for yourself. Here is an example for SCID:

- **R**emove T cells from patient
- **C**lone normal gene for ADA
- **D**isable retrovirus
- **A**dd retrovirus and clone gene
- **I**nfect T cell with retrovirus
- **R**eturn T cell with normal ADA gene back to patient.

Here is the mnemonic device: **R**un **C**arefully **D**own **A**n **I**nteresting **R**oad.

Make flashcards of your mnemonic devices to help you remember the many things you must memorize in biology.

Use of viral vector to treat SCID

The first human gene therapy was carried out in 1990 on a 4-year-old patient named Ashanti DaSilva. She had a genetic disorder called severe combined immunodeficiency (SCID). Patients with SCID lack a functional immune system. They have a genetic defect in gene ADA. ADA produces an enzyme that helps to metabolize another molecule, dATP. The defect in ADA results in a lack of the enzyme. Lack of the enzyme prevents metabolism (use) of dATP. Thus dATP builds up and, as you can imagine, causes lots of problems. All of this dATP is toxic to certain cells of the immune system called T cells. SCID therefore results in loss of T cells. We need T cells because they are helper cells for the B cells in the immune system. The B cells need T cells in order to recognize foreign invading cells and make antibodies against them. Without this functioning immune system, most patients with SCID die by the time they are teenagers. The following treatment was given to Ashanti and after 2 years she was showing near-normal T-cell counts.

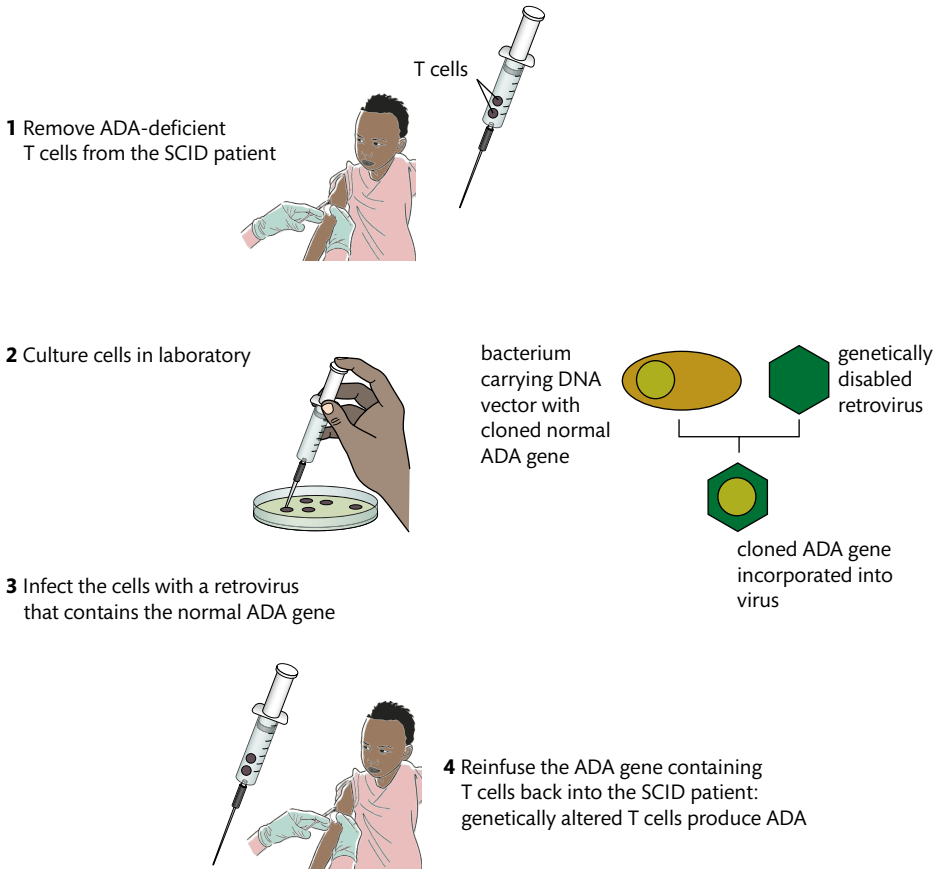


Figure 13.36 Gene therapy for SCID.
Thieman and Palladino 2013, p. 284

CHALLENGE YOURSELF

41 Look at the diagram of the gene therapy for SCID (Figure 13.36) and learn the steps. List the steps using numbers or bullets in your own words. Be specific and factual.

Jesse Gelsinger, who was 18 years old, died during a gene therapy clinical trial. He was the first person to die as result of gene therapy treatment. The discussion about the safety of gene therapy greatly intensified because of his death.

As you can see from Figure 13.36, the same procedures that have been mentioned already were used for the first gene therapy.

Risk is an inescapable reality of clinical research. In recent years, because of the widely reported occurrence of serious events, the fear of gene therapy has been heightened. What attention should be given to how decisions about risk are made by both researchers and their subjects?

TOK

Exercises

10 Outline the steps for using a DNA microarray.

11 Explain how PCR is carried out.

12 List three metabolites found in blood and urine that indicate disease.

NATURE OF SCIENCE

Cooperation and collaboration between groups of scientists: databases on the internet allow scientists free access to information.

B.5 Bioinformatics

Understandings:

- Databases allow scientists easy access to information.
- The body of data stored in databases is increasing exponentially.
- BLAST searches can identify similar sequences in different organisms.
- Gene function can be studied using model organisms with similar sequences.
- Sequence alignment software allows comparisons of sequences from different organisms.
- BLASTn allows nucleotide sequence alignment while BLASTp allows protein alignment.
- Databases can be searched to compare newly identified sequences with sequences of known function in other organisms.
- Multiple sequence alignment is used in the study of phylogenetics.
- EST is an expressed sequence tag that can be used to identify potential genes.

Applications and skills:

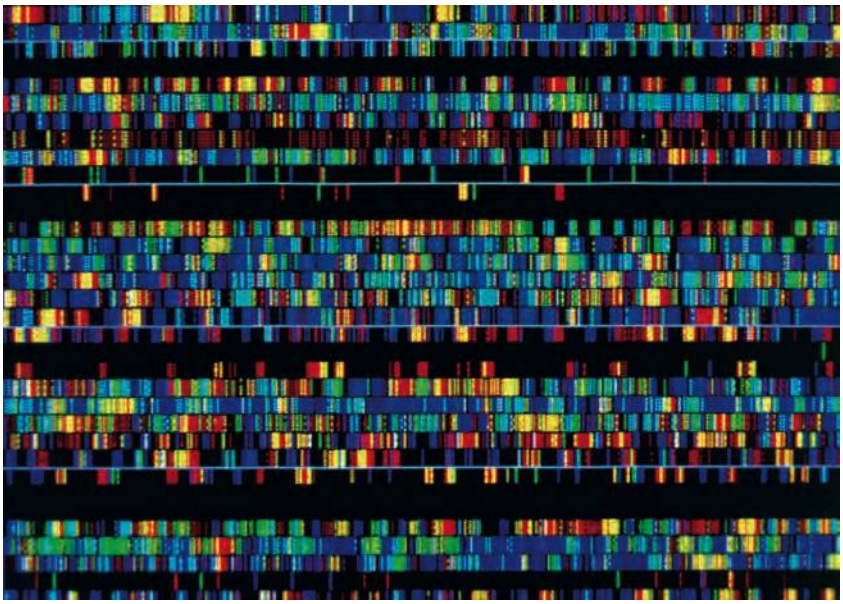
- Application: Use of knockout technology in mice to determine gene function.
- Application: Discovery of genes by EST data mining.
- Skill: Explore chromosome 21 in databases (for example in Ensembl).
- Skill: Use of software to align two proteins.
- Use of software to construct simple cladograms and phylograms of related organisms using DNA sequences.

Databases

For the first time in the history of biology, students can work along with other researchers at the same time using the same databases. These databases allow scientists easy access to information. Amazingly, this access is available to everyone: the databases are public information. Today, experiments that were previously conducted *in vivo* (in live cells) are now conducted 'in silico' (with a computer). These databases are ready for you to use as you begin to explore the subject of bioinformatics.

Bioinformatics is a research field that uses both computer science and information technology to help us understand biological processes. Bioinformatics has grown exponentially in the past decade. The most data-rich area of bioinformatics is genomics.

The Human Genome Project has given us much of the genomics of the human genome. It was completed in 2003 and is a map of the entire human genome, with all of the bases (ATGC) placed in the proper order and all of the genes located on the correct chromosome. The human genome data and that of the sequencing of many other species are now available in public databases such as The National Center for Biotechnology Information (NCBI).



The human DNA sequence

Stored data is increasing exponentially

An incredible amount of data is being generated in biology. This increasing data is an invaluable resource for the biological community. There are well-established databases like GenBank, but also small specific databases are emerging. There are organism-specific databases like FlyBase and WormBase. There are databases of protein families and databases built around disease. As the volume of biological data grows, so do the number and types of databases.

Four major databases are:

- Swiss-Prot, a well-curated database of protein sequences
- Ensembl, a database and browser of genomic information about humans and other vertebrates
- GenBank, a National Institutes of Health genetic sequence database that is an annotated collection of all publicly available DNA sequences
- OMIM (On-Line Mendelian Inheritance in Man), a description of phenotypes for a series of disease-causing SNPs (mutations in the human genome).

Five elements to a good database are:

- an accession code (a unique identifier)
- the name of the depositor (the name of the scientist who discovered the information and put it in the database)
- deposition data, indicating when the data were entered in the database
- a literature review, providing information on articles that have been written about the data so that more information can be gathered
- the real data, so that the actual data are in the database and not held by a private company.

Bioinformation is kept in safety in databases. The databases are modern cyber-safe museums or reference collections, where knowledge is carefully classified and saved. We will be using some of these databases to see exactly how much information has been stored for scientists and the public to use freely.

NATURE OF SCIENCE

One community-curated (curated by scientists of the scientific community globally) database is called Metabase (MB). The aim of this website is to help researchers find the databases that they need. The entire scientific community is encouraged to contribute to the maintenance of this site. Similar to a 'wiki' space, this is an example of collaboration and cooperation between groups of scientists.

BLAST

A biological database is an organized body of data usually supported by a computerized software program. You use this software to search the data and analyse it. An example of software that we will use is BLAST. BLAST is the software used to search the database GenBank. GenBank is the largest public database of DNA sequences. It works like this.

- A scientist clones a gene.
- The scientist enters the sequence of the gene into GenBank.
- GenBank checks the sequence of the gene against other sequences to see if there is a match. The series of letters is called FASTA information.
- The result gives the scientist information about what organism(s) has the same gene, the name of the gene, and the function of the gene.

BLAST is the acronym for Basic Local Alignment Search Tool. It searches the database GenBank for local alignments. BLAST is a 'local' alignment tool, which means that it does not attempt to align the whole length of the sequence being searched but searches only for regions of similarity. Local alignment can detect small regions of similarity, which may be more biologically significant than larger regions.

BLAST searches can identify similar sequences in different organisms

Similar sequences are often found in different organisms. An example is the human and mouse. There are alignments between many genes of the mouse and humans. One particular gene that we will look at is the gene for obesity. The mouse gene sequence for obesity (ob) produces the hormone leptin, which is involved in fat metabolism. Mutations in the leptin gene can contribute to obesity.

Let us use the BLAST website to find information about the human gene for leptin and see how closely it is aligned with the same gene in a mouse.

- Go to the BLAST website: www.pearsonhotlinks.co.uk/url.aspx?urlid=68764 (or do an internet search for 'Blast').
- Choose a BLAST program to run: nucleotide blast.
- In the large rectangle carefully type in these nucleotides in lower case letters. There are 60 letters to this part of the sequence that you are typing. Here they are: gtcaccaggatcaatgacatttcacacacgaaatcagtctcctccaaacagaaagtcacc
- Scroll to the bottom of the page and click on BLAST.
- Wait a few minutes and your results will appear.
- Scroll past the coloured graph down to the DESCRIPTIONS.
- Under descriptions go to the column called IDENT (which means % of identity) and scroll down to 86% identity. Here we can see that the mouse, *Mus musculus*, gene is 86% identical to the human gene for leptin. These two organisms share a core set of genes, so that experiments with mice can give us information about human genes. You might note the higher and lower % identities of different organisms on the list.
- Next to the % is the Accession code for this gene, HQ166716.1. Click on the Accession code and a window will open that will give you lots of information about the gene. (An accession code is given to each entry in GenBank for reference purposes.) It tells you the name of the organism: *Mus musculus*.

- Scroll down the left-hand column to Authors. The authors who put the information about the gene into the database are named: Hong, C.J. *et al.*
- Scroll down to PUBMED. Click on the number next to PUBMED and you will go to an Abstract about the mouse gene.

This explains a small amount of the information you can see using BLAST. To make you even more familiar with BLAST, go back to the BLAST website.

- Click on: nucleotide blast.
- Type in the Accession code for a gene: U14680.
- Click BLAST.
- Read the Description. The gene is *Homo sapiens* breast and ovarian susceptibility gene (BRCA1).
- Click on Query ID.
- Scroll down the left-hand column to PUBMED.
- Click on PUBMED. Notice at the top of the page that the journal is *Science*. The date of the article is 1994. The abstract states that the BRCA1 gene has been identified.
- Return to the previous page. Scroll down to ORIGIN and you will see the entire nucleotide sequence (cDNA) of this gene.

Much more information about the BRCA1 gene is available on this website for scientists or students to use. For example on the right-hand side of the page you will find several articles about the BRAC1 gene. Click on the title and you will see the article.

The more you explore BLAST or any of the other websites we will use, the more you will learn about them. There are lots of YouTube tutorials for BLAST online.

BLAST is a type of sequence alignment software

What you have just done with BLAST is to align a sequence of nucleotides to a similar sequence of nucleotides in different organisms. Sequence alignment software can align nucleotides or can align proteins. Why would a scientist want to align sequences of DNA, RNA, or proteins? The reason could be to find any:

- functional relationship, for example genes for leptin have the same function in a mouse or a human (we have just demonstrated that in the worked example above)
- structural relationship, for example if a scientist has isolated a protein but does not know what its function is, it can be structurally aligned in a database with another protein and the function may be learned (we will be looking at this below)
- evolutionary relationship, for example to find common ancestors and show phylogenic relationships (we will be looking at this below).

BLASTn and BLASTp

Nucleotide BLAST (BLASTn) aligns nucleotide sequences, just as we did when we typed the human gene for leptin into GenBank. Using BLASTn, we found the alignment with nucleotide sequences of other organisms in the database, such as the mouse. We can do the same thing using protein BLAST (BLASTp). BLASTp compares the sequence of a protein by aligning the sequences of amino acids that make up the protein (peptide). The FASTA information that you inserted in the large rectangular Query box in BLASTn (nucleotide BLAST) was a series of letters. What the letters of FASTA stand for in a BLASTn or BLASTp can be seen in Tables 13.5 and 13.6.

Table 13.5 Nucleic acid codes (used in BLASTn)

Nucleic acid code	Meaning	Mnemonic
A	A	**A**denine
C	C	**C**ytosine
G	G	**G**uanine
T	T	**T**hymine
U	U	**U**racil
R	A or G	pu**R**ine
Y	C, T or U	p**Y**rimidines
K	G, T or U	Bases that are **K**etones
M	A or C	Bases with a**M**ino groups
S	C or G	**S**trong interaction
W	A, T or U	**W**eak interaction
B	Not A (i.e. C, G, T or U)	**B** comes after A
D	Not C (i.e. A, G, T or U)	**D** comes after C
H	Not G (i.e., A, C, T or U)	**H** comes after G
V	Neither T nor U (i.e. A, C or G)	**V** comes after U
N	A C G T U	a**N**y
X	Masked	
–	Gap of indeterminate length	

Comparing a newly identified sequence with a sequence of known function in another organism

Now let us do a protein alignment activity. For our hypothetical (pretend) newly identified protein sequence, use the letters of the name of one of the authors of your textbook as the FASTA sequence. We can make up the sequence because it is newly identified. In fact, after this activity you can try making the newly identified protein sequence your name. Here is how to begin.

- Go to the BLAST website.
- Under basic BLAST click on protein blast.
- On the page there is a large rectangular box that says Query Sequence above it. Below it you can enter the accession number of the FASTA sequence. (FASTA is the letter codes that stand for the amino acids in a peptide). We will use the author's maiden name as a substitute for a peptide.
- Type in carefully: patriciamarygallagher.
- Scroll down and click BLAST. Be patient and eventually you will see a window with Protein Sequence 21 letters at the top.
- First there is a graph, Distribution of 100 BLAST hits on the Query sequence. Scroll down past this until you get to DESCRIPTIONS.

Table 13.6 Amino acid codes (use for BLASTp)
The following codes are for 24 amino acids and three special codes.

Amino acid code	Meaning
A	Alanine
B	Aspartic acid or asparagine
C	Cysteine
D	Aspartic acid
E	Glutamic acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
O	Pyrrolysine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
U	Selenocysteine
V	Valine
W	Tryptophan
Y	Tyrosine
Z	Glutamic acid or glutamine
X	Any
*	Translation stop
–	Gap of indeterminate length

- The best alignments are at the top. Let's look at the top line. Notice the name is murid herpesvirus 4. Click on the accession code at the end of that first line. It will take you to a separate page devoted to that protein. On this page there is a lot of information valuable to researchers.

REFERENCE: this tells you the names of the authors who have put information about this protein in the database. In this case there are three references.

PUBMED: click on the number next to PUBMED and you will be taken to an article published by Reference 1 authors. Go to the PUBMED article. What is the function of this protein? Read the first sentence of the abstract and you will see that it is a virus that infects mice.

- Return to the previous page. Scroll down to ORIGIN, this shows you the entire sequence of the protein. Each letter represents an amino acid in the protein. You can find what the letters mean by looking up the chart on amino acid codes.

Now that you are familiar with BLASTp, insert your name (first, middle, and surname) into the FASTA query rectangle. Just remember that the letters, Z, B, J, O, U, and X, do not occur in protein sequences. If those letters are in your name, just skip them. Now your name is a hypothetical newly identified sequence. What is the function of this newly identified sequence? Use BLASTp to find out.

Use of software to construct simple cladograms and phylograms

TOK

Should we worry about the claims of scientists using different databases? Is the knowledge offered by researchers using different methods to collect their data from different databases equally justified?

Now we will use a database to find out about evolutionary relationships. We know that organisms that share many features are closely related and probably had a relatively recent common ancestor. The features that scientists originally compared were physical features. They looked to see whether an organism had hair or feathers, legs or wings. Based on those structures they drew a diagram of relatedness called a phylogenic tree or dendrogram (see Figure 13.37). We now know that comparisons of the sequence differences in either proteins or nucleic acids can be more exact for indicating relatedness.

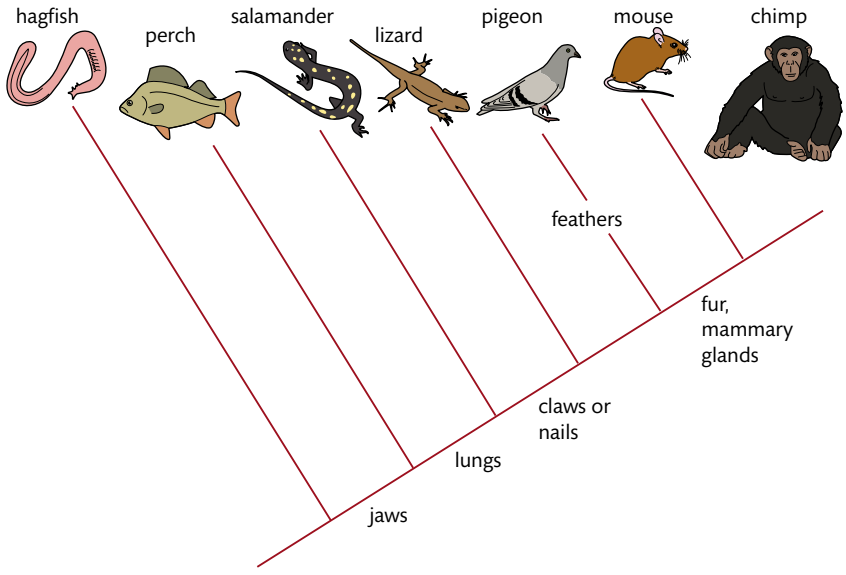


Figure 13.37 A phylogenic tree of vertebrate chordates. Purves et al. 1998

Using modern bioinformatic tools, we can compare nucleic acid and protein databases from many organisms to examine their evolutionary relationships. The software can make a cladogram or phylogram for us. A cladogram is a type of phylogenic tree in which

the lengths of the edges do not represent evolutionary time. A phylogram is a type of phylogenic tree in which the lengths of the edges do represent evolutionary time.

In this example, we will use a protein found in many organisms called haemoglobin. You may remember this protein from studying about the human body. Haemoglobin is the oxygen-carrying molecule in our blood. It contains four protein chains, two alpha and two beta. You may also remember that sickle cell anaemia is caused by one amino acid that has only one mutation in each of the two beta chains. We will explore the relationship between eight organisms by using the small changes that are present in their beta haemoglobin chains as a basis for comparison. Finally the software will output a phylogram or cladogram showing the pattern of how they are related.

Here are the eight organisms:

- domestic duck
- alligator
- human
- rat
- Canada goose
- Nile crocodile
- rhesus monkey
- mouse.

A Nile crocodile hatchling.

In order to compare these haemoglobin beta chains, we need to have the protein sequences for each organism. To find the protein sequence we must go to the database called Swiss-Prot.

- Go to this website to find Swiss-Prot: www.pearsonhotlinks.co.uk/url.aspx?urlid=68726 (or do an internet search for 'Swiss-Prot expasy').
- Click on Download – UniProt FTP sites.
- In the Query box type human beta haemoglobin.
- Click search. Next a large page of information will be displayed.
- Find the entry number for the entry name: HBB human (entry number P68871).
- Scroll down and look at all of the organisms that have beta haemoglobin. There are seven pages of organisms.
- Go back to the HBB human entry P68871 and click on it.
- You will see a page that is only about HBB human. It contains Names and origin, Protein attributes, General annotation, and many more headings. Keep scrolling down until you finally arrive at Sequences (just before References). Notice that the 147 letters correspond to the letters of the chart of amino acid codes used for BLASTp. If you copy the sequence on your clipboard and save it, then you will have it for the next activity.

Now that you know how to find the Sequence for a human, you can find the other sequences at the following website or the Word document on your eText: www.pearsonhotlinks.co.uk/url.aspx?urlid=68727

- Click on the Hemoglobin File link. It contains the haemoglobin beta chain downloads for all eight organisms.
- Open the haemoglobin file. Copy the entire sequence for all eight organisms onto your clipboard.
- Go to the website we will be using for multiple sequence alignment: Clustal Omega www.pearsonhotlinks.co.uk/url.aspx?urlid=68728 or do an internet search for 'Clustal Omega'. This is a bioinformatics website that is held at the European Bioinformatics Institute.
- Go to your downloaded haemoglobin file and select all the text and paste it into the text box in the rectangle just below STEP 1 (enter your input sequences).
- Scroll down and click on SUBMIT.

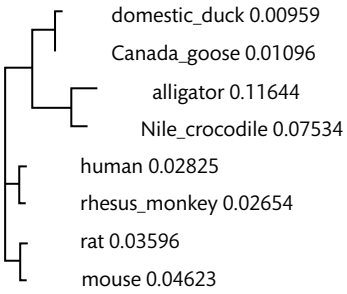


Figure 13.38 Cladogram showing the common ancestry of eight organisms. This common ancestry has been discovered by using a database that compares small differences in the haemoglobin beta chain of each organism.

Clustal Omega performs multiple alignments. It aligns more than two sequences at the same time. It is now aligning all eight sequences that we have submitted. Eventually it will cluster them on a tree-like diagram (a cladogram or phylogram).

The page that comes up is the alignment page.

- At the top in the tabs, select the tab Phylogenetic tree (if you can't see this, try using a different browser).
- Scroll down to Phylogram. The result is the cladogram shown in Figure 13.38. The branch length of the cladogram represents the amount of evolutionary divergence. The longer the branch, the greater the divergence.
- Click on Real, next to Branch length. Notice that the branch length is the same for each animal (Figure 13.39). A cladogram only shows common ancestry and does not include the length of divergence.

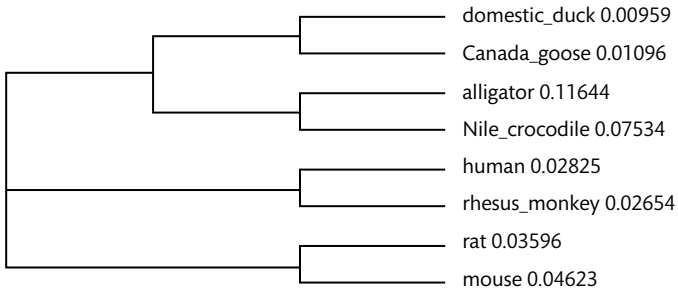


Figure 13.39 Cladogram showing the amount of evolutionary divergence of eight organisms using the same information as we used for the cladogram in **Figure 13.38**. The longer the branch, the greater the divergence.

NATURE OF SCIENCE

A perfect example of scientists cooperating and collaborating together is Ensembl. The European Bioinformatics Institute (EBI) and the Sanger Institute, both located near Cambridge, UK, have cooperated to develop an integrated software and database system of genomic information. This system is a resource for all the researchers studying the genomes of humans, other vertebrates, and model organisms.

Exploring chromosome 21 in the database Ensembl

Another database we can use is Ensembl. Ensembl matches proteins to the position of the DNA that codes for a protein on a chromosome. It is a database that predicts gene location and displays it. The Ensembl project is a public database accessible to everyone, including you. Researchers add to this database when they discover, by experimentation, where a certain gene is located on a certain chromosome. Every day more information is added to this database, and every day other scientists access the information that is already there to help them in their research projects. Let's try it.

Use the following steps to go to the website and explore chromosome 21. Remember from your study of genetics that an extra chromosome 21 (trisomy) is the cause of Down syndrome. Scientists are very interested in chromosome 21 because of that syndrome.

- Go to www.pearsonhotlinks.co.uk/url.aspx?urlid=68729, or do an internet search for Ensembl.
- Click human.
- You will find five light-blue rectangles of information.
- Click on Vega.
- One blue rectangle is named Annotation progress.
- Click on chromosome 21.
- From here you can explore chromosome 21 by either zooming in on a part of a chromosome or by looking at the chart of chromosome statistics.

Many tutorials are available for Ensembl if you want to explore further.

EST data mining

Researchers are still finding out the function of the many genes that were located during the Human Genome Project. The EST database is a place where these DNA sections can be put together, like the pieces of a puzzle. EST stands for expressed sequence tag. EST can be used to identify potential genes. An EST is a short piece of cDNA (copied DNA) sequence that is single-stranded. It can be used to discover genes or to determine the sequence of a gene. An EST is a small fragment of cDNA typically present in a DNA library. ESTs can be:

- mapped to a certain chromosome location
- or, if the gene containing the EST has been sequenced, it can align the EST to that genome.

ESTs have recently become an important tool that is helping us understand the function of human genes.

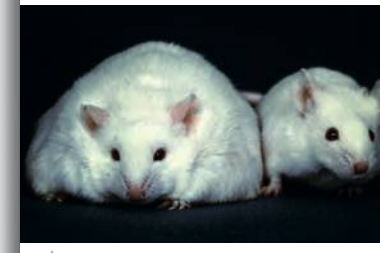
A recent study using EST data analysis discovered three genes expressed in human prostate cancer. The procedure involved searching the EST sequences from human prostate cDNA libraries. Clones of selected ESTs were tested and the results analysed using a computer program. Fifteen promising genes were identified that were previously unknown. Seven of these genes were examined in a hybridization experiment and three were found to be prostate specific. These three genes can now be used in the targeted therapy of prostate cancer.

The bank of sequencing computers at the Sanger Center.

Knockout technology can help determine gene function

We have seen that gene function can be studied using a model organism. The mouse, *Mus musculus*, was the organism we used when looking at the leptin gene that is conserved in humans and many other organisms (an identical DNA sequence that occurs across species). We used a database for comparison, but another method to determine exactly what a gene does is to 'knock it out' and see what happens. The mouse is a common knockout (KO) species. Researchers have knocked out the leptin gene in mice by replacing it with a mutant gene, and found that the mice become obese.

Why do researchers commonly use mice? The mouse is genetically and physiologically similar to humans, and its genome can be easily manipulated and analysed. Diseases affecting humans, such as cancer and diabetes, also affect mice. Even if the mouse does not normally have a disease (e.g. cystic fibrosis), it can be induced to have it by manipulating its genome. Adding to the appeal is the low cost of mice and their ability to multiply quickly. Many inbred strains and genetically engineered mutants are available for researchers. Important advances in genetic technology have given us the tools to knock out mice genes, which means replacing normal genes with alternative versions. See Table 13.7 for some examples.



An obese mouse (knockout mouse with mutant gene Lep <ob>) unable to produce leptin next to a normal mouse.

Leptin comes from the Greek word 'leptos' which means thin.

Table 13.7 Examples of knockout mice

Knockout mouse	Defect	Benefit to research
Cftr	Defective in the gene that makes CFTR, a protein that regulates passage of salts and water in and out of cells	Allows research into cystic fibrosis, which is the most common fatal genetic disease in the USA
P53	Has a disabled Trp53 tumour gene	Cancer research
Lep<ob>	Has a mutant gene for leptin	Obesity research

Other model organisms used in comparative genomics

Complete sequences of the model organisms shown in Table 13.8 have been found and added to databases. In some research these organisms are as important or even more important than the mouse.

Table 13.8 Examples of model organisms

Model organism	Group
Escherichia coli	Bacteria
Arabidopsis thaliana	Plant
Saccharomycetes cerevisiae	Yeast
Drosophila melanogaster	Insect
Caenorhabditis elegans	Nematode
Mus musculus	Mammal

The number of genes that we share with these species is very high. It ranges from a 30% similarity with yeast to an 80% similarity with mice.

The honeybee genome has been completed and is being used to help the honeybee industry understand bee genetics

To find out more about bioinformatics, go to the hotlinks site, search for the title or ISBN, and click on Chapter 13: Section B.5.

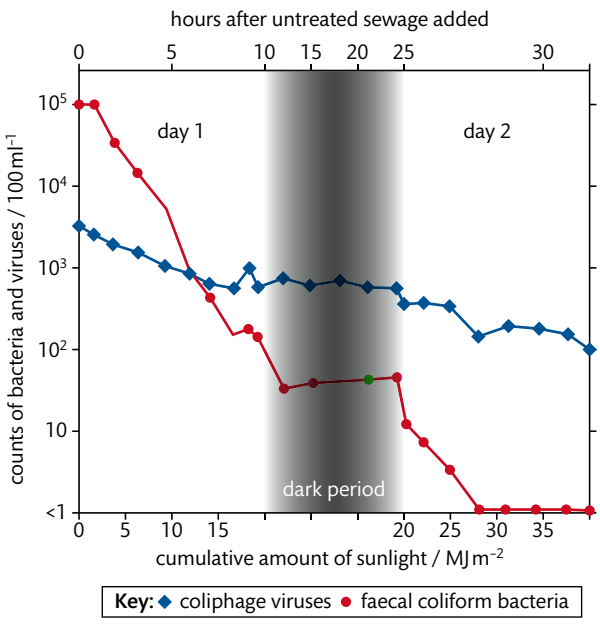
Exercises

13 Compare and contrast BLASTn and BLASTp.

14 List three reasons for using sequence alignment software.

15 Explain the benefit of knockout mice.

Practice questions



Adapted from Sinton 1999

1 Release of sewage in marine waters is a common practice but it can cause water contamination with pathogens. A series of experiments was conducted to compare inactivation rates of two different groups of microbes with different sunlight exposures. One group was faecal coliform bacteria and the other was coliphage viruses. Experiments were conducted outdoors using 300-l mixtures of sewage–seawater in open-top tanks.

A 2-day experiment was carried out with untreated sewage added to seawater. Both days were sunny with no clouds. The figure below shows the inactivation of the microbes in seawater as a function of the cumulative amount of sunlight and time. The survival curves of the two microbes are plotted against sunlight exposure (lower *x*-axis) during daylight periods and against time during the overnight period (upper *x*-axis). The *y*-axis gives counts of bacteria and viruses per 100 ml.

(a) Identify the time at which faecal coliform bacteria counts fell below 1 unit per 100 ml. (1)

(b) Deduce, using the data in the graph, the effect of sunlight on

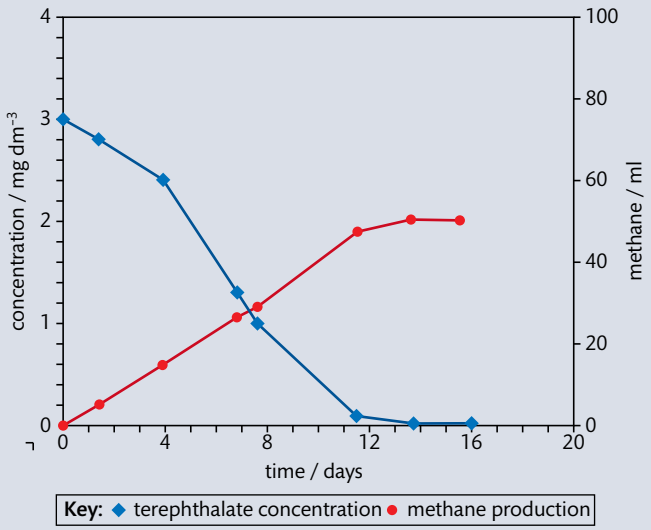
(i) faecal coliform bacteria (2)

(ii) coliphage viruses. (2)

(c) For an accidental sewage spill, suggest, giving a reason, which of the two microbes may be most useful as a faecal indicator 2 days after the spill. (1)

(*Total 6 marks*)

2 Waste water from factories producing polyester fibres contains high concentrations of the chemical terephthalate. Removal of this compound can be achieved by certain bacteria. The graph below shows the relationship between breakdown of terephthalate and conversion into methane by these bacteria in an experimental reactor.



Adapted from Wu et al. 2001

(a) The reactor has a volume of 12 l. Calculate the initial amount of terephthalate in the reactor. (1)

(b) Describe the relationship between terephthalate concentration and methane production. (2)

(c) Suggest which bacteria can be used for the degradation of terephthalate. (1)

(d) Evaluate the efficiency of the terephthalate breakdown into methane. (2)

(*Total 6 marks*)

3 **(a)** Outline the use of a viral vector in gene therapy. (3)

(b) Discuss the risks involved in gene therapy. (2)

(*Total 5 marks*)

14 Option C: Ecology and conservation

Essential ideas

C.1	Community structure is an emergent property of an ecosystem.
C.2	Changes in community structure affect and are affected by organisms.
C.3	Human activities impact on ecosystem function.
C.4	Entire communities need to be conserved in order to preserve biodiversity.
C.5	Dynamic biological processes impact population density and population growth.
C.6	Soil cycles are subject to disruption.

The cane toad, *Rhinella marina*. The cane toad was brought to Australia in the 1930s in an unsuccessful attempt to control the population of beetles that were eating the sugar cane crop.

A community is a group of populations living together and interacting with each other in an area. The community might be named by an environmental feature, for example a sand dune community or pond community. Other communities might be named after the dominant plant species, such as an oak community or a redwood forest community.

The distribution of organisms in communities is affected by both abiotic (non-living) and biotic (living) features. We are interested in studying all these factors to determine what may affect a certain population of organisms. For example, does the fact that cars drive up to the beach with their lights on affect sea turtle reproduction at that beach? Is the rabbit population in a forest decreasing because of an increase in the fox population?

The study of populations tells us about the factors that affect all the living things around us. It can be used to predict what might happen to, for example, populations of fish in the marine environment if we continue to overfish. The population growth model of a simple organism like yeast can show us a typical pattern that can be applied to other populations. If we want to know how many animals are in a population, we can estimate the population size by using the technique of capture–mark–release–recapture.

Biodiversity is nature's 'backup': it is the reserve needed to survive a disaster. A disaster could wipe out the majority of organisms, but leave the few that are the best adapted. In order to know how much biodiversity exists in an ecosystem, we must be able to measure it. The Simpson index of biodiversity is one method of measuring diversity.

The soil is a key factor in the health and growth of all populations. Nitrogen and phosphorous are key nutrients in the soil. We know that animals eat the plants that depend on the soil for these nutrients. The cycles of these nutrients are basic to the lives of all living things. Nitrogen is recycled from the soil to plants, then to animals and back to the soil. Phosphorus is much less available than nitrogen. It is only present in the soil as a result of the weathering of sedimentary rock. The unavailability of phosphorous levels may become a future limiting factor for agriculture.

NATURE OF SCIENCE

Use models as representations of the real world: zones of stress and limits of tolerance graphs are models of the real world that have predictive power and explain community structure.

C.1 Species and communities

Understandings:

- The distribution of species is affected by limiting factors.
- Community structure can be strongly affected by keystone species.
- Each species plays a unique role within a community because of the unique combination of its spatial habitat and interactions with other species.
- Interactions between species in a community can be classified according to their effect.
- Two species cannot survive indefinitely in the same habitat if their niches are identical.

Applications and skills:

- Application: Distribution of one animal and one plant species to illustrate limits of tolerance and zones of stress.
- Application: Local examples to illustrate the range of ways in which species can interact within a community.
- Application: The symbiotic relationship between zooxanthellae and reef-building coral reef species.
- Skill: Analysis of a data set that illustrates the distinction between fundamental and realized niche.
- Skill: Use of a transect to correlate the distribution of plant and animal species with an abiotic variable.

Limiting factors affect the distribution of species in a community

Why do you live where you live? Is it because it's near public transport? Is it where your family has always lived? Is it because of its proximity to a school? Some factors limit where we choose to live. Other organisms have factors that limit where they live because of necessity, not choice. The distribution of a species depends on these factors, which are both abiotic (non-living) and biotic (living).

Abiotic factors include:

- light, for example day length and intensity
- atmosphere, for example the amount of CO_2, O_2, or N_2 in the atmosphere
- water availability
- temperature
- salinity of soil or water
- soil conditions, for example pH, aeration, soil composition (loam, clay, or coarse soil), soil components such as the amount of humus, sand, or rock.

Biotic factors include:

- living organisms in the ecosystem
- inter-relationships of organisms, such as symbiosis, parasitism, or predator–prey relationships, and competition for resources of food, water, and mates.

Use a mnemonic device to remember the list of abiotic and biotic factors: LAWs To Save Soil help us Live In the world.

The distribution of species depends on their tolerance of limiting factors

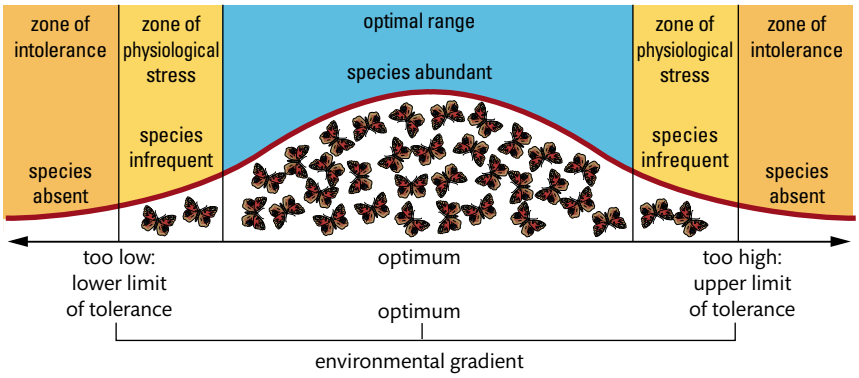


Figure 14.1 The law of tolerance states that distribution of species in an ecosystem is determined by the limits of physical and chemical factors that can be tolerated.

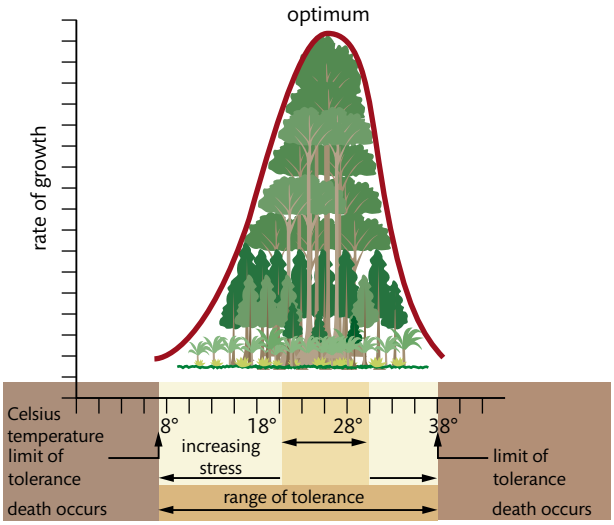


Figure 14.2 For every factor influencing growth, reproduction, and survival, there is an optimum level. Above and below the optimum, there is increasing stress, until survival becomes impossible at the limits of tolerance.
http://apesnature.homestead.com/chapter2.html

NATURE OF SCIENCE

Hypothetical curves illustrating zones of stress and tolerance can help us understand what is occurring in the real world.

The limits of tolerance and stress for organisms in an ecosystem have been described by an ecologist called Victor Shelford. Shelford's law of tolerance states that the levels of one or more chemical or physical factors determine the abundance and distribution of a species in an ecosystem. When the factors fall below or rise above the levels tolerated by the species, that species will cease to exist in that ecosystem.

Limits of tolerance and zones of stress: an animal example

Kangaroos in Australia are a good example of how climate can be a limiting factor in the distribution of species. This can be seen in Figure 14.3.

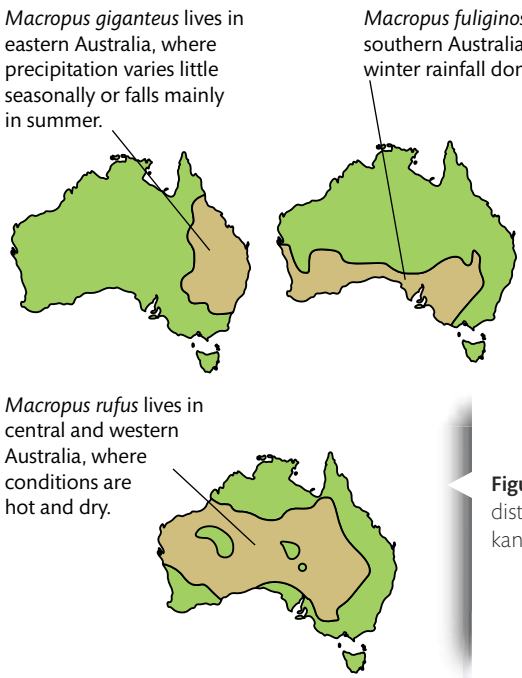


Figure 14.3 Climate and distribution of three different kangaroo species.

This distribution of kangaroos in Australia has been stable for over a century. The red kangaroo, *Macropus rufus*, lives in the arid and semi-arid interior of Australia. The distribution of the red kangaroo reflects the interaction of the mean annual temperature and the mean annual precipitation. Look at the graphs in Figure 14.4.

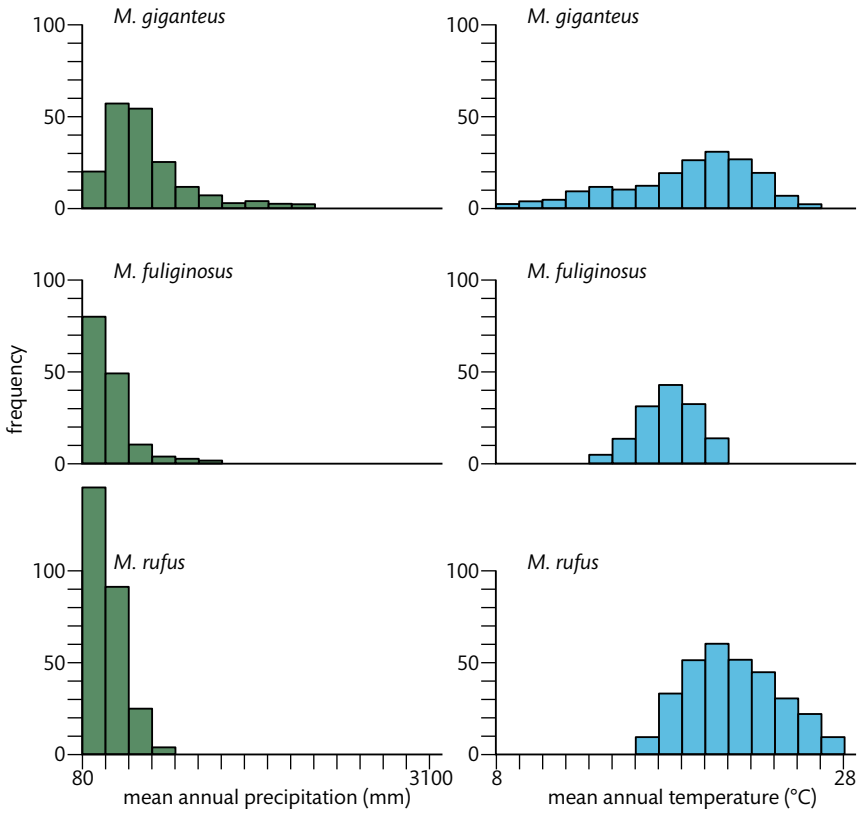


Figure 14.4 Three species of kangaroo have different limits of tolerance to precipitation and temperature. Caughley et al. 1987

You can see from the graphs that the red kangaroo is tolerant of much lower mean precipitation and higher mean temperatures than the other two species. This affects its distribution and its success in the difficult, arid, and hot interior of Australia, which is dominated by desert and savannah. Red kangaroos can live where the mean annual rainfall can be as low as 80 mm and the temperature can be as high as 40°C. This species of kangaroo possesses certain adaptations that increase its tolerance to extremes of high temperature and low moisture. For example, the lighter fur colour of the red kangaroo reflects sunlight better than the dark fur of the other kangaroo species. The nasal openings of the red kangaroo are larger than the other kangaroos, which increases its evaporative cooling ability. The kidneys of the red kangaroo conserve water by producing more concentrated urine. These reasons explain the limits of tolerance of the red kangaroo and how they differ from the other kangaroos in Australia.

You may notice on maps of the distribution of kangaroos in Australia that no kangaroos live in the north. Research has determined that the north is probably too hot for the eastern grey kangaroo, *Macropus giganteus*, too dry in winter for the western grey kangaroo, *Macropus fuliginosus*, and too wet for the red kangaroo. This supports the idea that the dry and hot limits of the distribution of the red kangaroo represent the levels to which it is well adapted.

Limits of tolerance and zones of stress: a plant example

A genus of plant along the coast of California, USA, shows a distribution pattern based on its level of tolerance to extreme environmental conditions. The plant is a shrub in the genus *Encelia*.

Encelia frutescens	
	
Scientific classification	
Kingdom:	Plantae
Phylum:	Angiospermophyta
	Eudicots
	Asterids
Order:	Asterales
Family:	Asteraceae
Genus:	*Encelia*
Species:	*E. frutescens*
Binomial name	
Encelia frutescens	

Encelia frutescens is a species of flowering plant in the daisy family known by the common names button brittlebush and bush encelia.

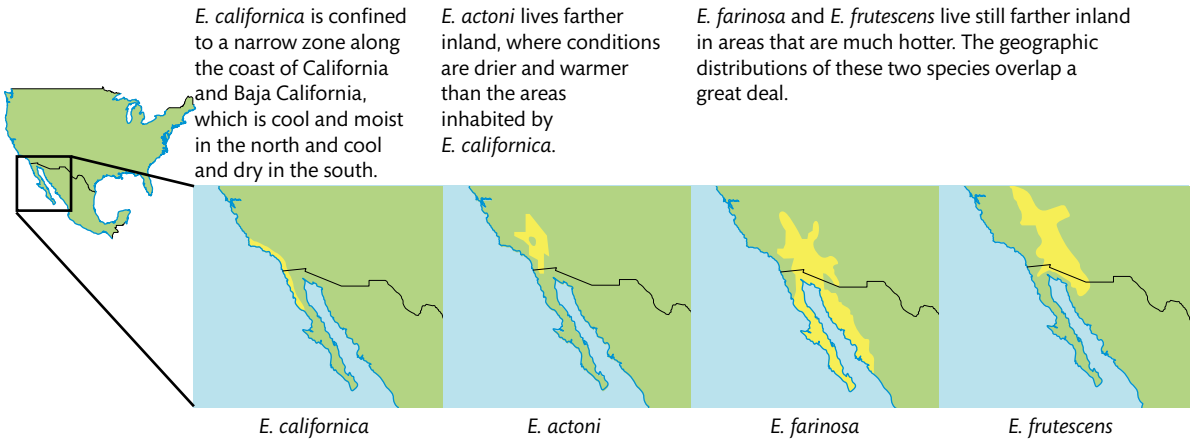


Figure 14.5 The distribution of four *Encelia* species.

Look at Figure 14.5, which shows the distribution of four *Encelia* species. The species we will be looking at is *E. frutescens*, the button brittlebush. It lives in the hottest and driest area on the coast. Rainfall can vary there from 100 mm to 400 mm yr^{-1}, and, compared with the other coastal areas pictured, the temperature range of *E. frutescens* is very hot, from 35 to 40°C. When we study the physiology of *E. frutescens*, we can see that is has adaptations that have enabled it to live in this extreme range of heat and dryness. It does not overheat because it has leaves that transpire at a high rate and are cooled by evaporation as a result. You might wonder how it gets enough water to cool its leaves by evaporation in one of the hottest and driest deserts in the world: notice that its microenvironment is limited to streambeds and desert washes. Along these beds, run-off water soaks into the soil, increasing the ability of the deep roots of *E. frutescens* to reach moisture. The limiting factor is in fact the availability of water in the streambeds and desert washes that it can access with its long root system. Because it has leaves that are not protected with hairs, it cannot survive dryness. Its nearest neighbour, *E. farinosa*, has pubescent leaves, which are leaves that are covered with hundreds of small hairs. These hairs allow *E. farinosa* to live on slopes in shallow soils that contain limited water. The hairs trap water so that its transpiration is limited, and they provide protection from the wind that would otherwise dry out the surface of the plant. *E. frutescens* cannot survive on the slopes with less water because it has no surface hairs on its leaves and needs water to replace the water lost by transpiration.

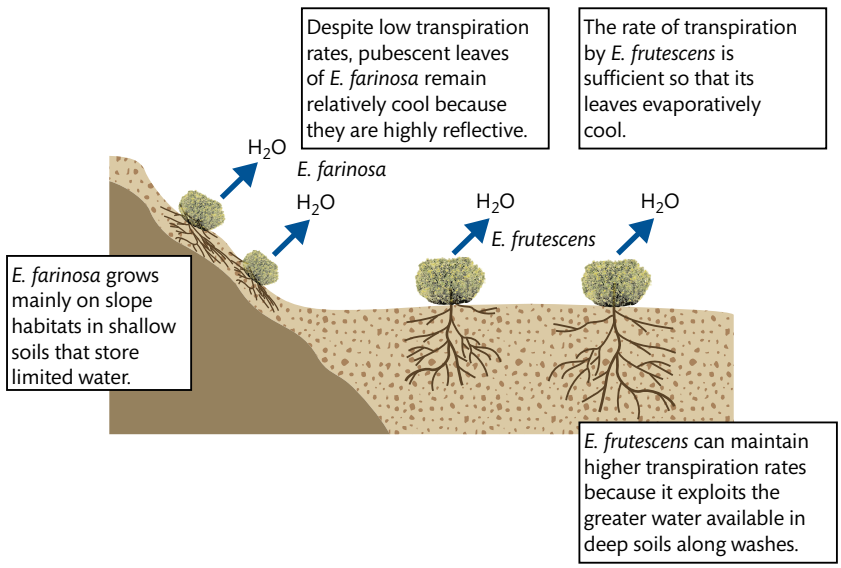


Figure 14.6 Temperature regulation and distribution. Molles 2010, p. 207, Fig. 9.7

Flowers can be mistaken for buds until you look closely.

TOK

Random samples are taken to study the numbers of plants in a certain area. Are data obtained from random samples justified?

The geographical range of *E. frutescens* is regulated by both temperature and moisture.

Keystone species

A keystone species is one that is not necessarily abundant but exhibits a strong control over the structure of a community. How do we determine which organism is the keystone species?

A good method to determine whether an organism is a keystone species is to perform a removal experiment. Ecologist Robert Paine first attempted this method. He was studying an intertidal area of western North America. When Paine removed the sea star, *Pisaster ochraceous*, manually from the intertidal area, a mussel, *Mytilus californianus*, was able to take over the rocky area and exclude algae and other invertebrates from that zone (see Figure 14.7). The mussel simply took over the space available when there was no sea star to keep it in check. It was evident that it was the sea star that limited the number of mussels that could reproduce and attach to the rocks. Paine collected data that showed that, when sea stars were present, 15–20 different species of invertebrates and algae were present. Without the sea star, the diversity rapidly declined to five species. This supported the hypothesis that the sea star was the keystone species. When it was present, it had control over the diversity of the community. When it was absent, the diversity was lost.

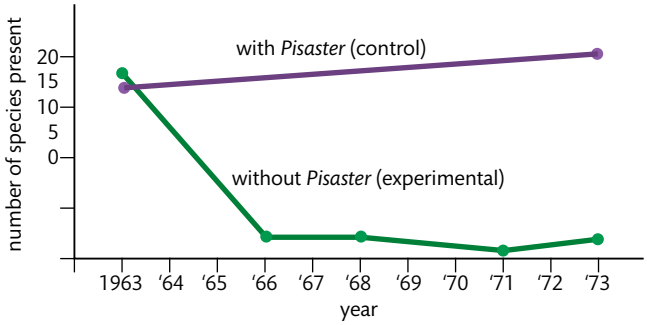


Figure 14.7 Testing a keystone species hypothesis. The effect of removing the sea star *Pisaster ochraceous* manually from an intertidal area over a 10-year period. Campbell and Reece 2002, Fig. 53.14

CHALLENGE YOURSELF

Look at Figure 14.8.

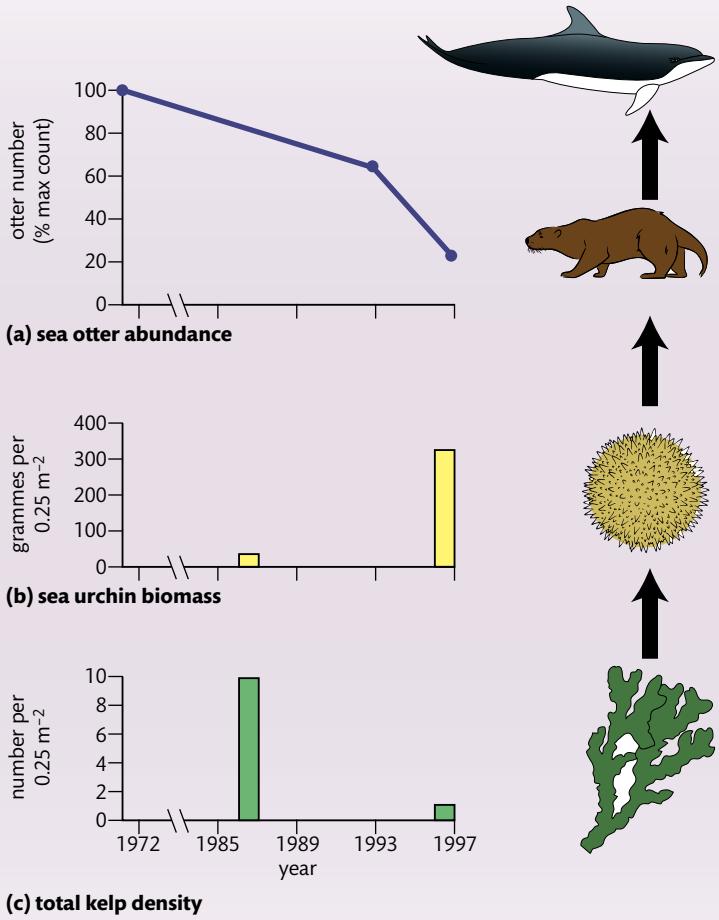


Figure 14.8 A food chain in the North Pacific of kelp–sea urchin–sea otter–orca. Adapted from Campbell and Reece 2002, Fig. 53.15

1 Look at the food chain shown on the sides of the graphs. Which organism would you hypothesize is the keystone species?
2 Use the graphs and explain why the data support your hypothesis.
3 The imbalance over 20 years is probably caused by the decline in seals and sea lions, which are also food for orca. Can you think of a reason for the decline in seals and sea lions?

Each species plays a unique role within a community

The unique role that a species plays in the community is called its niche. A famous ecologist, Eugene Odum, once said 'If an organism's habitat is its address, the niche is the habitat plus its occupation.' We could put it another way and say that the concept of niche includes where the organism lives (its spatial habitat), what and how it eats (its feeding activities), and its interactions with other species.

Spatial habitat

Every type of organism has a unique space in the ecosystem. The area inhabited by any particular organism is its spatial habitat. The ecosystem is changed by the presence of the organism. For example, leopard frogs, *Rana pipiens*, live in the ponds of Indiana (USA) dunes. They burrow in the mud in between the grasses on the edge of the pond.

Feeding activities

The feeding activities of an organism affect the ecosystem by keeping other populations in check. For example, the leopard frogs in the Indiana dunes eat the aquatic larvae of mosquitoes, dragonflies, and black flies. The presence of the leopard frog helps keep the populations of these insects in check.

Interactions with other species

The interactions of an organism with other species living in its ecosystem include competition, herbivory, predation, parasitism, and mutualism. The organism may be in competition with another organism for the food supply. It may itself be the prey for a larger predator. It may harbour parasites in its intestines. These complicated interactions are difficult to uncover, but they indicate the importance of the organism in the ecosystem. The predator of the green frog is the blue heron. Without the green frog in the sand dune ecosystem, the heron would have a significantly reduced food supply. Frogs are homes for flatworm parasites that live in their intestines. Without doubt there are many other relationships between the green frog and other species.

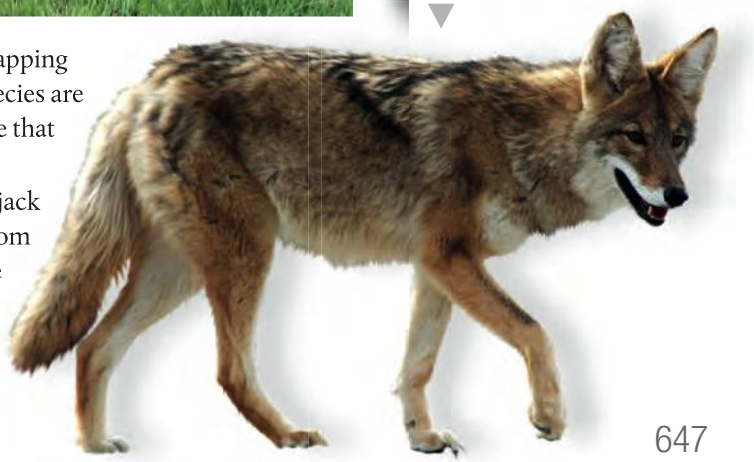
One of the jobs of an ecologist is to collect data on the niches of particular organisms in an ecosystem. If an organism is in danger of becoming extinct in an ecosystem, it is necessary to understand as many of its interactions as possible in an attempt to determine the cause of its extinction. What follows now are some explanations and examples of interactions between species.

Competition

When two species rely on the same limited resource, one species will be better adapted than the other to benefit from the resource.

- Example 1: In the USA, coyotes, *Canis latrans*, and red foxes, *Vulpes vulpes*, are both predators that eat small rodents and birds. Coyotes inhabit grassland communities in the USA, while the red fox prefers the edges of forests and meadows. Because more farmland has been created and more forests removed, the habitat of the red fox is disappearing and is overlapping with that of the coyote in the grasslands. The two species are competing for a smaller food supply and it is possible that one will become extinct in that habitat.
- Example 2: In the coastal dunes of the UK, the natterjack toad, *Epidalea calamita*, is facing tough competition from the common toad, *Bufo bufo*. Disturbance of the dune area is limiting the habitat available to both toads.

The habitat of the red fox is disappearing.



The coyote is competing with the red fox for a small food supply. Removal of forests and creation of farmland has eliminated some of their food supply.

Herbivory

A herbivore is a primary consumer (plant eater) that feeds on a producer (plant). The growth of the producer is critical to the well-being of the primary consumer. This is an interaction between plants and animals.

- Example 1: Rabbits, *Oryctolagus cuniculus*, eat marram grass in a sand dune ecosystem.
- Example 2: The monarch butterfly, *Asclepias syriaca*, larvae eat the leaves of the milkweed plant.

Canadian lynx walking through deep snow tracking an Arctic hare.

Predation

A predator is a consumer (animal) eating another consumer (animal). One consumer is the predator and another is the prey. The number of prey affects the number of predators and vice versa.

- Example 1: The Canadian lynx, *Lynx canadensis*, and the arctic hare, *Lepus articus*, form a classic example of predator–prey interaction. The lynx preys on the hare. Changes in the numbers of the lynx population are followed by changes in the numbers of the hare population.
- Example 2: The blue heron, *Ardea herodias*, is a predator on frogs in the ponds of American sand dune ecosystems.

Parasitism

A parasite is an organism that lives on or in a host and depends on the host for food for at least part of its life cycle. The host can be harmed by the parasite.

- Example 1: *Plasmodium* is a parasite that causes malaria in humans. It reproduces in the human liver and red blood cells. Part of the life cycle of the *Plasmodium* takes place in the body of the *Anopheles* mosquito. The mosquito is the vector that transmits the malaria parasite from one human to another.
- Example 2: Leeches, *Hirudo medicinalis*, are parasites that live in ponds. Their hosts are humans and other mammals. Leeches puncture the skin of a host and secrete an enzyme into the wound to prevent clotting. Leeches can ingest several times their weight in blood.

Mutualism

Two species living together where both organisms benefit from the relationship is termed mutualism.

- Example 1: Lichen is a mutualistic relationship between algae and fungi. The algae, *Trebouxia*, photosynthesize and make carbohydrates (food) that the fungi can use. The fungi, mainly *Ascomycota* species, absorb mineral ions needed and used by the algae.
- Example 2: *Rhizobium* is a nitrogen-fixing bacterium that lives in the roots of leguminous plants such as beans and peas. *Rhizobium* fixes nitrogen (transforms atmospheric nitrogen into a form that is useable by plants), which the plant can then use to make proteins. The plant makes carbohydrates (during photosynthesis), which can be used as food by the *Rhizobium*.
- Example 3: Clownfish, *Amphiprion ocellaris*, and sea anemones, *Anemonia sulcata*, live together for mutual benefit. Clownfish are small brightly coloured fish that live within the area of the tentacles of the poisonous sea anemone. The clownfish is covered with mucus that protects it from the sting of the sea anemone. Clownfish

lure other fish to the waiting tentacles of the sea anemone. After the sea anemone kills the fish, the clownfish and the sea anemone both eat the remains. The clownfish also nibble off the remains of dead sea anemone tentacles.

Cavernous star coral, *Montastraea cavernosa*. The greenish colour on the coral is zooxanthellae algae.

- Example 4: Zooxanthellae are single-celled algae that live in the tissue of reef-building coral. The coral provides the compounds and the environment for photosynthesis for zooxanthellae. In turn, the algae provide food for the coral. The algae give the coral a boost of nutrients so that it can secrete the skeleton of calcium carbonate that it needs to build the reef. This is a highly efficient exchange of nutrients in a nutrient-poor environment. This relationship of mutual benefit is called mutualism or symbiosis, living together for mutual benefit.

CHALLENGE YOURSELF

4 What interactions between species have you learned about in this section? List four or five types of interactions and give an example of each.

The relationship between zooanthellae and coral is a type of symbiosis. The coral and algae live together. 'Bio' is the Greek word for living, and 'sym', is the Greek word for together.

Competitive exclusion

You will recall that the red fox and coyote may now be in competition with each other for resources. They seem to both hunt for their food in the same areas, and the food supply may be dwindling as a result of the forests and grasslands being turned into farmland. If the fox and the coyote do begin to occupy the same niche in the ecosystem, the principle of competitive exclusion can be used to predict the end result.

The principle of competitive exclusion states that no two species in a community can occupy the same niche.

In 1934, the competitive exclusion principle was demonstrated by a Russian ecologist, G. F. Gause. He performed a laboratory experiment with two different species of *Paramecium*: *P. aurelia* and *P. caudatum* (see Figure 14.9). His experiments showed the effects of interspecific competition between two closely related organisms. When each species was grown in a separate culture, with the addition of bacteria for food, they did equally well. When the two were cultured together, with a constant food supply, *P. caudatum* died out and *P. aurelia* survived. *P. aurelia* out-competed *P. caudatum*. The experiment supported the Gausian hypothesis of competitive exclusion. When two species have a similar need for the same resources, one will be excluded. One species will die out in that ecosystem and the other will survive. *P. aurelia* must have had a slight advantage that allowed it to out-compete *P. caudatum*.

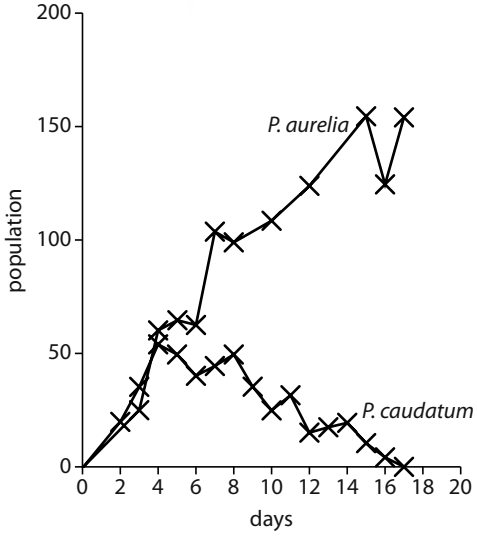


Figure 14.9 Competitive exclusion.

Fundamental niche versus realized niche

The red fox's habitat in the USA is the forest edge. Its food consists of small mammals, amphibians, and insects. It interacts with other species, such as the mosquitoes that suck its blood and scavengers that eat its leftovers. This is the fundamental niche of the red fox. The fundamental niche is the complete range of biological and physical conditions under which an organism can live.

What has happened to the red fox's fundamental niche? The forest edge has been turned into farmland in many places. Some of the species eaten by the red fox have disappeared. The red fox must survive in a narrower range of environmental conditions. Now there is direct competition from the coyote, whose niche has also been changed. This new and narrower niche is called the realized niche.

Make sure to label all parts of a graph: the title, *x*-axis, *y*-axis, units, and uncertainties.

The fundamental niche of a species is the potential mode of existence, given the adaptations of the species.

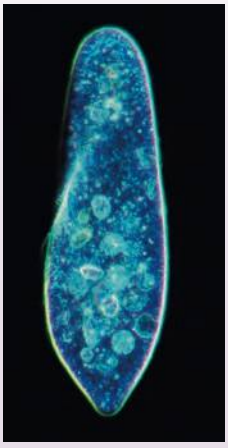
The realized niche of a species is the actual mode of existence, which results from its adaptations and competition with other species.

CHALLENGE YOURSELF

Paramecium caudatum is a single-celled organism that lives in fresh water. In an experiment researchers allowed *P. caudatum* to grow for 28 days in order to determine its normal growth curve. Every 7 days, ten random samples were collected from the population. The data recorded are shown in Table 14.1

Table 14.1 Random samples of the population density (number per mm^3, ±5 organisms) of a culture of *P. caudatum* taken over 28 days

Sample number	Day 7	Day 14	Day 21	Day 28
1	143	200	300	390
2	155	205	315	360
3	165	185	295	375
4	135	235	350	365
5	143	195	295	410
6	145	265	320	370
7	165	265	340	380
8	175	195	370	390
9	105	215	325	390
10	169	290	340	320
Mean				



Paramecium caudatum, seen under a light microscope.

5 Calculate the means of the data and graph them.

6 Will this give you a picture of the fundamental or realized niche? Give your hypothesis as to what the results will show.

A second experiment was performed where *P. caudatum* was placed in a culture with another species of *Paramecium*. The researchers wanted to know, when the two species are competing for resources, what will be the result?

Tables 14.2 and 14.3 show the data that were collected over 28 days.

Table 14.2 Random samples of the population density (number per mm³, ±5 organisms) of *P. caudatum* taken over 28 days

Sample number	Day 7	Day 14	Day 21	Day 28
1	169	290	340	300
2	105	215	325	315
3	175	195	370	295
4	165	265	340	350
5	145	265	320	295
6	143	195	295	320
7	135	235	350	340
8	165	185	295	370
9	155	205	315	325
10	143	200	300	340
Mean	150	225	325	325

(The means have been calculated for you.)

Table 14.3 Random samples of the population density (number per mm³, ±5 organisms) of *P. bursaria* taken over 28 days

Sample number	Day 7	Day 14	Day 21	Day 28
1	75	160	210	160
2	85	150	190	190
3	65	150	190	250
4	75	140	220	180
5	85	140	230	180
6	65	130	180	230
7	75	170	180	220
8	95	170	250	190
9	70	130	190	190
10	60	160	160	210
Mean	75	150	200	200

7 Graph the data from this experiment.

8 Is this graph showing the fundamental or realized niche of *P. caudatum*? Explain your answer.

Use of a transect to correlate the distribution of a plant with an abiotic variable

A transect is a method of sampling a population of plants or animals along a longitudinal section of an ecosystem. The observer moves along a fixed path to count the occurrences of the plant or animal along the path. It is much more accurate to use this type of transect with plants, because they do not move. Line transects are used to illustrate a particular gradient of an abiotic factor, such as sunlight or soil moisture, that is present in the ecosystem.

Marram grass.

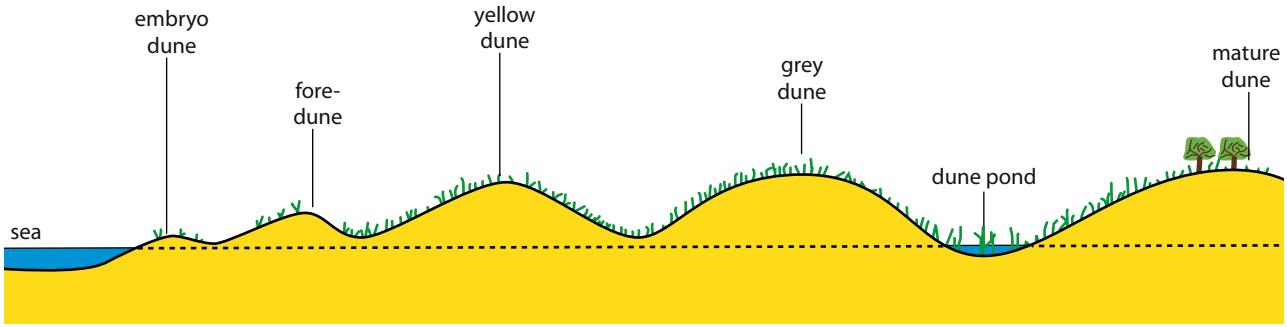


Figure 14.10 Transect of a coastal sand dune.

An example can be seen in Figure 14.10, showing the transect of a coastal dune. Let's look at the distribution of marram grass in the coastal dune ecosystem and how it is affected by the abiotic factor of soil pH.

If you were asked to do a transect, you would do the following.

- At right angles to the sea, lay a tape in a line all the way up the dunes.
- Every 10 or 20 m along the tape, mark out a quadrat (a square of a certain size).
- Identify and count the tufts of marram grass in the quadrat.
- Take several samples of the soil in each quadrat and use a soil test kit to determine the pH of the soil.
- Record all the data in a table
- Turn it in to a diagram of your choice.

You can now determine the pattern of distribution of marram grass from the youngest dune to the oldest dune and see if it correlates with changes in the soil pH.

Transect information can be very useful when making decisions about ecosystems that are important to us. Here is an example of how transects can be important in an ecosystem. In this example, an experiment was performed to determine whether artifical light would affect the foraging behaviour of salamanders in an ecosystem

Transects were used in forested areas at the Mountain Lake Biological Station in Virginia, USA. Half of the transects were lit by strings of white minilamps placed within the transects. The other half were not lit. The researchers walked each transect at night in order and counted the number of salamanders. There were significantly more active salamanders in the dark transects than in the light ones. The salamanders in the dark transects were foraging for food. The salamanders in artificial light were not foraging. This experiment shows how the use of artificial light to illuminate a campsite or even a research station can affect some organisms negatively.

To learn more about using sampling in fieldwork, and about using transects, go to the hotlinks site, search for the title or ISBN, and click on Chapter 14: Section C.1.

Exercises

1 Describe a method to determine whether an organism is a keystone species in an ecosystem.
2 Design an experiment using a transect to correlate the distribution of a plant with an abiotic factor.
3 Outline an example of symbiosis.

C.2 Communities and ecosystems

Understandings:

- Most species occupy different trophic levels in multiple food chains.
- A food web shows all the possible food chains in a community.
- The percentage of ingested energy converted to biomass is dependent on the respiration rate.
- The type of stable ecosystem that will emerge in an area is predictable based on climate.
- In closed ecosystems energy but not matter is exchanged with the surroundings.
- Disturbances influence the structure and rate of change within ecosystems.

Applications and skills:

- Application: Conversion ratio in sustainable food production practices.
- Application: Consideration of one example of how humans interfere with nutrient cycling.
- Skill: Comparison of pyramids of energy from different ecosystems.
- Skill: Analysis of a climograph showing the relationship between temperature, rainfall, and the type of ecosystem.
- Skill: Construction of Gersmehl diagrams to show the inter-relationships between nutrient stores and flows between taiga, desert, and tropical rainforest.
- Skill: Analysis of data showing primary succession.
- Skill: Investigation into the effect of an environmental disturbance on an ecosystem.

Guidance

- *Examples of aspects to investigate in the ecosystem could be species diversity, nutrient cycling, water movement, erosion, leaf area index, among others.*

NATURE OF SCIENCE

Use models as representations of the real world: pyramids of energy model the energy flow through ecosystems.

Energy flow through the ecosystem

What do you think is the direction of energy flow for any ecosystem? If you constructed a food chain like this one, then you know.

grass → cow → human

Plants are at the bottom of the food chain. They contain the highest amount of energy, which they obtain from sunlight. The source of energy for most ecosystems is the Sun. A few food chains are supported by bacteria that can trap chemical energy.

Only 5–20% of the Sun's energy that is trapped by plants is transferred to the primary consumers eating the plants. Why is this? Because 80–95% of the energy is lost as heat or used for maintenance by the plant. Energy is lost as heat as it moves from producer (e.g. grass) to primary consumer (e.g. a cow) to secondary consumer (e.g. a human).

This is the same reason why the fuel we put in a car is only partially used to run the car. A large percentage of the energy provided by the fuel is lost as heat. This is why there is a fan in the engine of a car. A law of physics called the second law of thermodynamics states that, when energy is transferred, a proportion of it is lost as heat energy. This law applies equally to cars and ecosystems.

Where is the energy from the Sun actually kept in the plant? Plants produce glucose during photosynthesis. Plants also break down the glucose molecules and use the energy released for maintenance activities. The breakdown is called respiration. Maintenance activities that need energy are growth, repair, and reproduction. When the glucose is used as fuel for these activities, some of the energy is lost. Some of the energy moves through the ecosystem as excretion. Some energy is left in

undigested food and is passed on to decomposers. When an organism dies, its body is decomposed and the energy transferred to decomposers.

Gross production, net production, and biomass

Pyramids of energy show how much energy is left at each trophic level (see Figure 14.11). Each block in the pyramid represents a trophic level (producers, primary consumers, secondary consumers, tertiary consumers). The width of the block indicates how much energy it contains. At each level, the blocks get narrower, and the block at the top is very narrow. The number at each level represents the amount of energy at each level. Can you see that only 10% of the energy from one trophic level is transferred to the next level? This diagram represents the ideal situation. In an actual ecosystem, the percentage transfer from one level to the next depends on many factors and may vary between 5% and 20%. In animal husbandry (farming), the transfer value is often higher than 10%. However, the loss of energy between producer and consumer explains why a kilogramme of beef is more expensive than a kilogramme of corn.

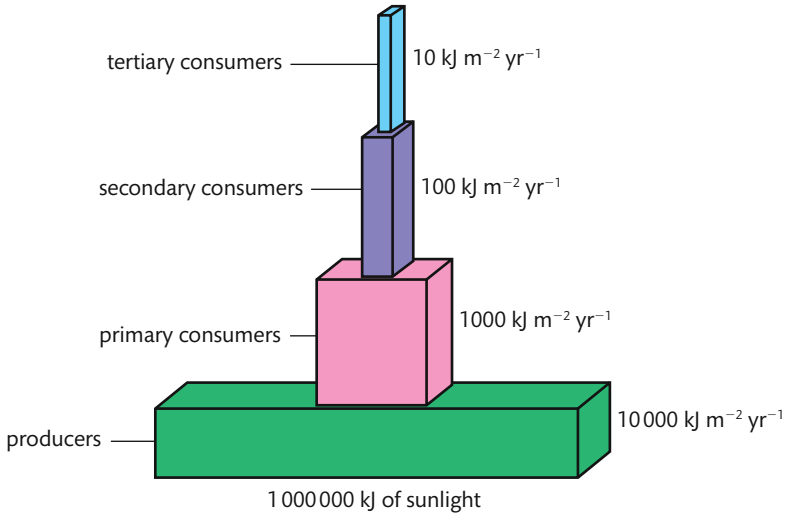


Figure 14.11 A pyramid of energy (not drawn to scale).

Figure 14.12 is a pyramid of energy with greater detail than the idealized pyramid shown in Figure 14.11. First, look at the simpler view at the bottom of the figure. The gross production of the producers is 20 810 kilojoules per metre squared per year (kJ m^{-2} yr^{-1}). Can you calculate what percentage of energy moved up to the herbivores?

Doing practice calculations like this will help you understand how this works.

About 16% of the energy moved up to herbivores. Now look at the detailed energy flowchart at the top half of Figure 14.12. Notice that 1 700 000 kJ of energy are input from the Sun and that only 1.2% of the Sun's energy was captured by the producers. The producers have a gross production of 20 810 kJ m^{-2} yr^{-1} . Gross production is the energy that they have available. Notice that some of that energy is lost as metabolic heat and net system loss (heat, respiration, and maintenance). Look on the other side of the figure, and you will see how much is transferred to 'organic wastes and remains'. This energy eventually flows through decomposers, like mould and bacteria in the soil, and detritivores, like earthworms. Calculate the percentage of energy that is lost as respiration (metabolic heat) as it moves to herbivores.

The answer is 63%. About 16% was transferred to herbivores and the rest was transferred to decomposers and detritivores. The energy reaching the carnivores is 11.4%, and only 5.5% flows up to the top carnivores. Look at the bottom of the energy

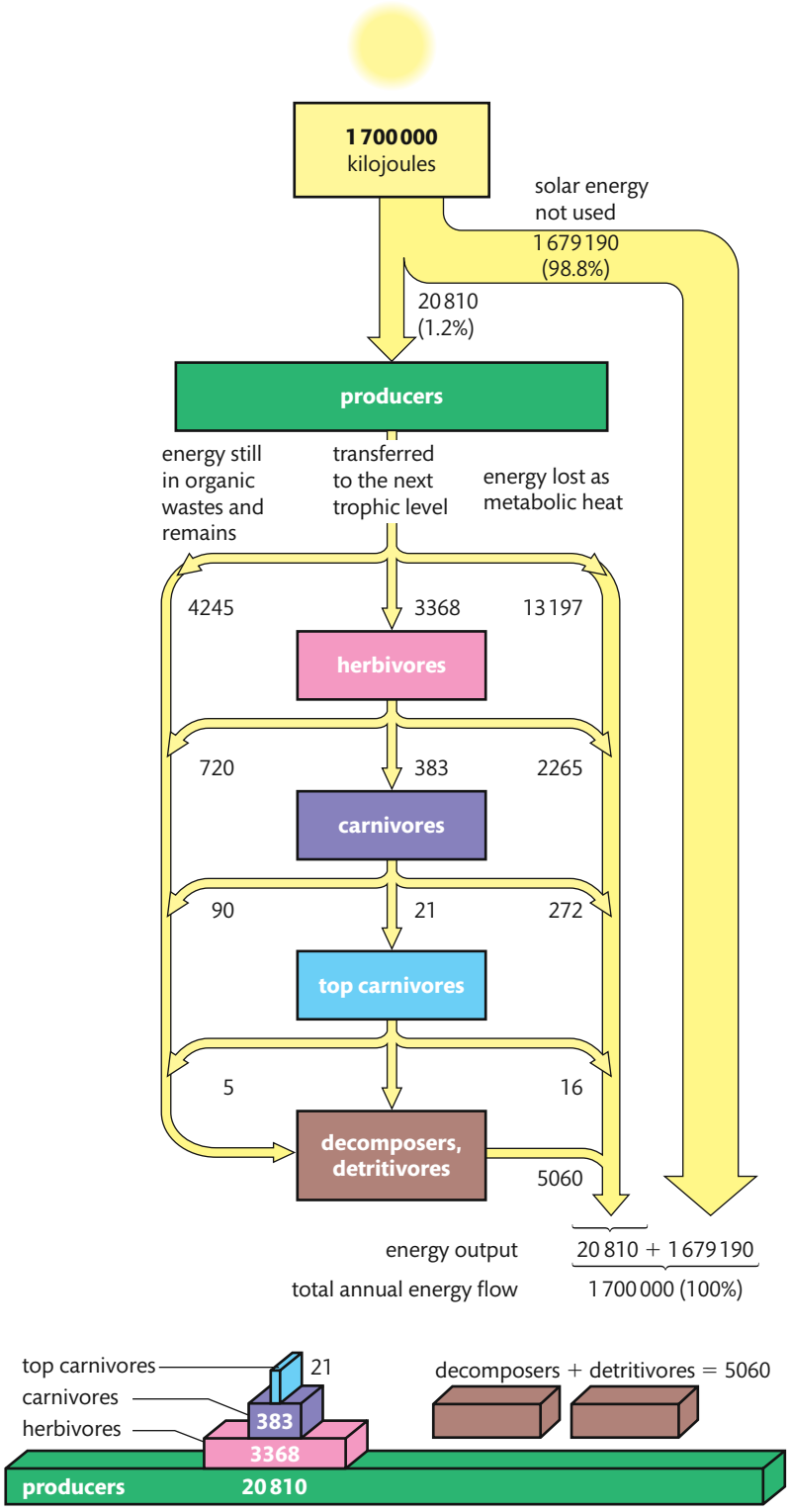


Figure 14.12 A pyramid of energy for the ecosystem in Silver Springs, Florida, USA measured in kJ m^{-2} yr^{-1}.

flow diagram and you will see that eventually all the energy that flows through the ecosystem is lost as metabolic heat.

However, within the specific time period covered by a diagram such as Figure 14.12, organisms are storing some of energy. For example, a young forest accumulates

organic matter as the tree grows. The slow rate of decay in a peat bog causes peat to build up. Some energy-flow diagrams include a cube to represent storage.

Now that you understand energy pyramids, we can define some important terms.

- Gross production is the total amount of energy trapped in the organic matter produced by plants per area per time in kilojoules, measured as kilojules per metre squared per year (kJ m^{-2} yr^{-1}).
- Net production is the gross production minus the energy lost through respiration, also measured as kJ m^{-2} yr^{-1}.
- Biomass is the dry weight of an organism, measured in grammes per metre squared per year (g m^{-2} yr^{-1}).

In terms of an ecosystem, biomass is the dry weight of all the organisms at a certain tier of an ecosystem. The reason why we use dry weight is that the actual weight of the organisms includes a large amount of water. Water needs to be removed and the dry weight measured.

Calculating gross production and net production

In order to calculate the values of gross production and net production, we use the equation:

$$\text{gross production} - \text{respiration} = \text{net production}$$

So, if:

$$\text{gross production} = 809 \text{ kJ } m^{-2} \text{ } yr^{-1}$$

and:

$$\text{respiration} = 729 \text{ kJ } m^{-2} \text{ } yr^{-1}$$

then:

$$\text{net production} = 80 \text{ kJ } m^{-2} \text{ } yr^{-1}$$

Constructing a pyramid of energy

Using the data below, construct a pyramid of energy without looking back at Figure 14.12.

Trophic level	Energy flow (kJ m^{-2} yr^{-1})
Producers	20 810
Primary consumers	3368
Secondary consumers	383
Tertiary consumers	21

After you have drawn the pyramid, check Figure 14.12 to see if yours is correct. Have you drawn each block in proportion to the numbers? Have you placed the correct labels at each trophic level? Have you remembered a title for your pyramid?

Pyramids of biomass

Pyramids of biomass are similar in shape to pyramids of energy. The higher trophic levels have a lower total biomass per unit area of ecosystem (see Figure 14.13). Biomass

is lost during respiration at each trophic level. When glucose is broken down for energy, it is converted into carbon dioxide gas and water. Carbon dioxide and water are excreted and the biomass of glucose is lost. Each successive level of the ecosystem loses more and more biomass. The energy per gramme of food does not decrease, but the total biomass of food is less at each trophic level. Notice in Figure 14.13 how little biomass is present in tertiary consumers compared with producers. It is very similar to what we saw when we looked at the pyramid of energy.

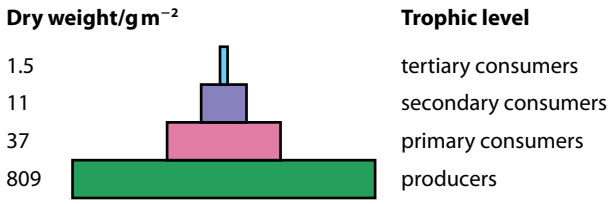


Figure 14.13 Pyramid of biomass.

A pyramid of numbers also has a similar shape as the pyramid of energy. Only a small amount of energy can flow all the way up to the highest trophic level. The total biomass of food available at the top trophic levels is also small. As the top predators, such as a shark or a lion, must be large enough to overwhelm their prey, there can be only relatively few of them.

Difficulties of classifying organisms into trophic levels

In order to understand the relationships of an ecosystem completely, something more than food chains and pyramids needs to be constructed. A food web gives a true but complicated picture of what is being eaten in an ecosystem (see Figure 14.14). Can you see the following difficulties when you look at the food web in Figure 14.14?

- An eagle is a tertiary consumer when eating rattlesnakes, but a secondary consumer when eating rabbits.
- A coyote is a primary consumer when it eats the fruit of a cactus, but a tertiary consumer when it eats a rattlesnake.
- A lizard is a tertiary consumer when it eats rattlesnake eggs, but a secondary consumer when it eats insects.

Another difficulty is where to put omnivores. For example, the following omnivores are difficult to classify into one trophic level.

- Grizzly bears eat plants, insects, and some mammals. Which food is eaten depends on the season, the temperature, and the bear's ability to forage for food. Are they primary consumers, secondary consumers, or tertiary consumers?

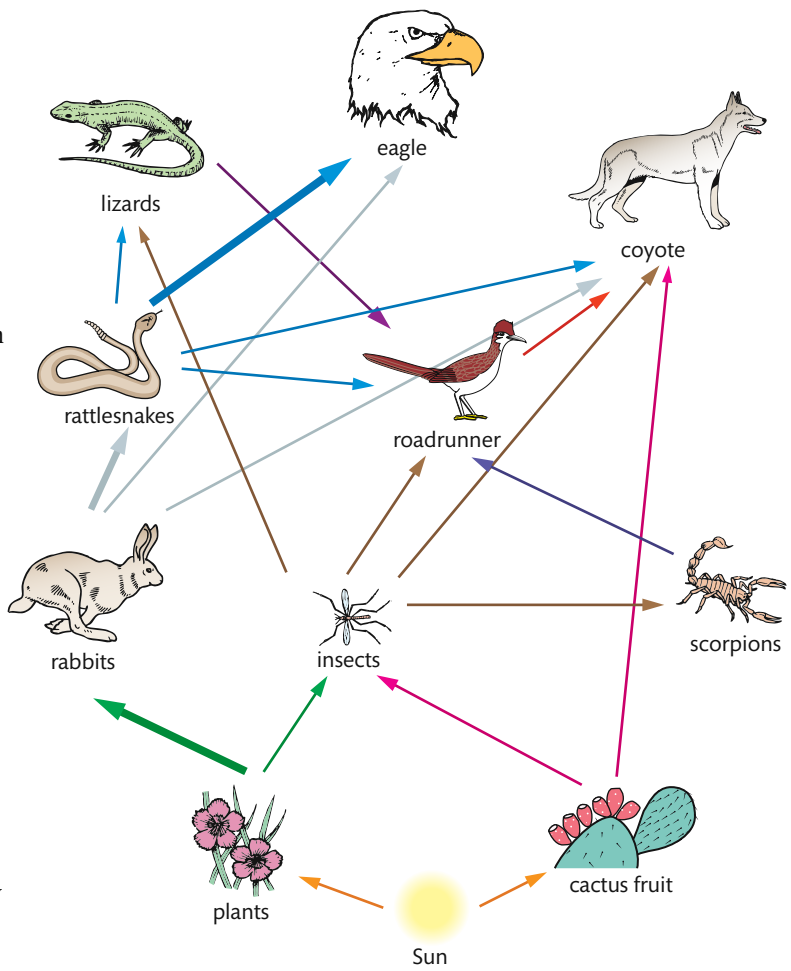


Figure 14.14 A desert food web.

TOK

Raymond Lindeman was an ecologist who formulated a new paradigm of energy flow through ecosystems. In addition to grouping organisms into primary producers, primary consumers, etc., he was the first scientist to measure trophic efficiency. Trophic efficiency is the production of one trophic level that is transferred to the next trophic level. This concept, first formulated in 1942, remains influential today.

- Raccoons eat mice, bird eggs, fish, frogs, nuts, and fruits. The food most dominant in the diet might depend on the season or competition from other animals. Is the raccoon mainly a primary consumer or a secondary consumer?
- Chimpanzees eat both fruit and termites. Is the chimpanzee mainly a primary consumer?

Comparing pyramids of energy

Table 14.4 A comparison of Cedar Bog and Lake Mendota, Wisconsin, USA

Trophic level	Cedar Bog		Lake Mendota	
	Productivity (cal cm^{-2} yr^{-1})	Efficiency (%)	Productivity (cal cm^{-2} yr^{-1})	Efficiency (%)
Solar radiation	119.000		119.000	
Plants	111	0.1	480	0.4
Herbivores	14.8	13.3	41.6	8.7
Carnivores	3.1	22.3	2.3	5.5
Higher carnivores			0.3	13.0

When comparing the energy pyramids of two different ecosystems you will notice that the difference is in their efficiency. Look Table 14.4 comparing the two lakes and you will see the transfer of energy at each trophic level. Notice that typically the organisms at higher and higher trophic levels are increasingly more efficient. Only a small percentage of the Sun's energy that plants absorb is available for transfer to the herbivores. Plants use up the energy through high assimilation and growth. Herbivores are slightly more efficient and carnivores are even more efficient. Cedar Bog has three trophic levels, while Lake Mendota has four. Five trophic levels are the limit for most systems. Lake Mendota can sustain another trophic level because it has a significantly larger biomass than Cedar Bog.

Here are two pyramids representing what we have just seen in Table 14.4.

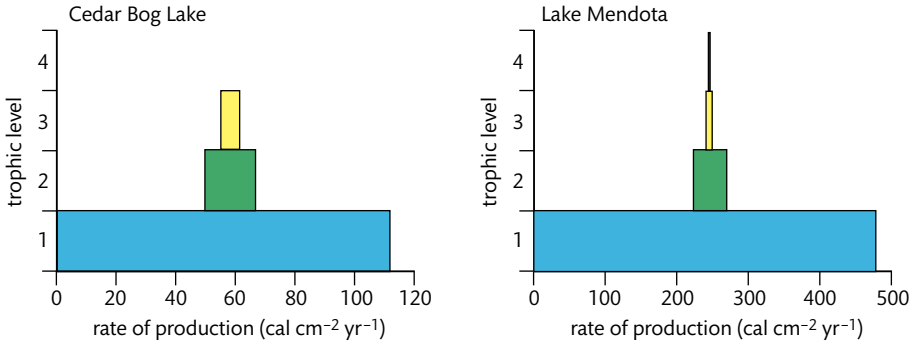


Figure 14.15 Annual production by trophic level in two lakes. Molles, Jr. 2010,Fig. 18.16

CHALLENGE YOURSELF

9 Referring to Table 14.4, what percentage of energy is passed on to the next trophic level from the plants of Cedar Bog to the herbivores of Cedar Bog?

10 What percentage of energy is passed on to the next trophic level from the plants of Lake Mendota to the herbivores of Lake Mendota?

Conversion ratio in sustainable food production

The feed conversion ratio (FCR) is a measure of the efficiency of an animal's ability to convert feed mass into increased body mass. It is expressed as a ratio:

$$\frac{\text{mass of food eaten}}{\text{body mass gain}}$$

For example:

$$\frac{8\text{ kg of food}}{1\text{ kg of weight gain}} = 8$$

Table 14.5 shows some estimates for farmed animals.

Table 14.5 Animal FCR

Animal	FCR
Cattle	5–8
Sheep	4–5
Pork	3
Poultry	2
Carnivorous fish (salmon)	2
Herbivorous fish (tilapia)	1.2–1.6

Animals with low FCR can be seen to be efficient users of food. The FCR shows us how much energy is being lost during the transfer from plant to animals, as we have seen with energy pyramids.

Can sustainable agriculture methods improve the FCR? The principles of sustainable agriculture are:

- maintenance of food safety
- improving the environment
- using resources efficiently
- improving the lives of families and society as a whole.

One practical example of sustainable food production is fish farming. Notice from Table 14.5 that fish have a very low FCR. Fish farmers are attempting to lower the FCR to 1. This would mean that the amount of feed given to the fish would be changed into 'fish mass' equal to the mass of feed. Therefore nothing would be lost and everything is gained, so long as other resources are not wasted.

A fish farm, Corfu, Greece.

Warm-blooded animals (homeotherms) are less efficient at converting food to biomass than cold-blooded animals (poikilotherms). So you can see why a fish is more efficient than a cow.

Change in ecosystems over time by primary and secondary succession

Ecological succession is the change in the abiotic (non-living) and biotic (living) factors in an ecosystem over time. It is the reason why some species gradually replace other species in one particular area.

Primary succession

Primary succession begins when plants begin growing on a previously barren and lifeless area. Let's consider a newly created volcanic island. The plants that first colonize it are able to exist where temperature changes are extreme and there is little or no soil. The first colonizers are usually lichens. They are pioneer plants that can decompose thin layers of rock. As they die and decompose, a thin layer of soil is formed. This is just enough for some moss to get a foothold. This is the start of primary succession. Eventually, there will be enough soil for other seeds to germinate. Coconuts may be washed ashore and begin to germinate. Coconut palm trees will grow. Animals may swim, fly, or be carried on floating vegetation from other islands and populate the new island.

Secondary succession

In secondary succession, a new group of organisms takes over following a natural or artificial upheaval of the primary succession. Secondary succession is much faster than primary succession because soil is already present and there may be existing seeds and roots present. Recolonization of an area after a forest fire is an example of secondary succession.

Table 14.6 summarizes the differences between primary and secondary succession.

The World Health Organization recently reported that more than 3 billion people are undernourished. This is the largest number and proportion of malnourished people ever recorded in history. The food shortage and malnourishment problem is primarily related to rapid population growth in the world plus a declining per capita availability of land, water, and energy resources.

Table 14.6 Primary and secondary succession

Primary succession	Secondary succession
Begins with no life	Follows a disturbance of primary succession
No soil	Soil is present
New area, e.g. a volcanic island	Old area, e.g. following a forest fire
Lichen and mosses begin to grow on volcanic rocks	Seeds and roots are already present
Biomass low	Biomass higher
Low production*	Higher production*

*Production is the increase in biomass or energy m^{-2} yr^{-1}. When production is low, it is because there are only a few plants; higher production occurs when many plants are present.

Species diversity and production in a primary succession

Coastal sand dunes are excellent examples of primary succession that are both interesting to walk through and have been studied extensively. If you do not live near the coast, use the hotlinks at the end of this section to find some resources. If you do live near the coast, you may find it more interesting to walk in the dunes after you have learned about the animals and plants that live there. Dunes are areas that need public support in order to be preserved as natural habitats.

Foredune

Primary succession starts on the foredune, where there is no soil, only sand. Lyme grass, *Leymus arenarius*, and marram grass, *Ammophila arenaria*, are pioneer plants on a new dune. Lyme grass is the more salt tolerant of the two species. It is generally fast growing and its roots help bind the sand and stabilize the dune. Marram grass has long underground roots that also spread sideways. It can spread 3 m yr^{-1}. Marram grass also has a special adaptation for life on a foredune: it has a growth spurt when covered with sand. There is little diversity of plant life on the foredune.

Yellow dune

At the yellow dune stage, the dune is developing a thin layer of soil from years of marram grass plants living and dying there. It has now been invaded by other plants with roots that are even better at binding the sand. These plants are sand sedge and

sand bindweed. Rabbits may be common in this dune, and their droppings add nutrients to the soil. In the summer, fast-growing plants like dandelions and thistles grow here. Humus (organic matter in the soil) begins to build up as the original pioneer plants die and decay. Notice that, at this stage, the community is more complicated. More species are present and soil is beginning to form.

Grey dune

The grey dune stage has developed a layer of humus from years of plants dying and decomposing. Humus holds water. This dune is much farther inland and sand is not deposited here. Eventually, thick shrubs will grow on this dune.

Mature dune

The final stage in dune succession is the mature dune, which can support a forest. At the Indiana dunes, the mature dune has an oak–hickory forest. Hundreds of species of wild flowers are protected by the shade of the trees. Mosses and ferns grow on the forest floor. The humus is thick as a result of 200 years of plants dying and decaying. The moisture content of the soil is high because of the high amount of humus. The forest is full of insects, birds, and mammals. The temperature is 10% cooler on the mature dune than on the foredune. Lack of wind and blowing sand makes this a comfortable place for both animals and plants.

During the development of the primary succession on sand dunes, you can see that the following changes have occurred:

- few species to many species
- pioneer species to species that compete with others for nutrients
- little diversity to high diversity, the mature forest is home to hundreds of different species
- simple relationships to more complex relationships of mutualism, competition, and predation
- more and more biomass at each stage of the succession.

A stable ecosystem will emerge based on climate

Ecological succession will occur until finally it develops enough complexity to become a stable community. The type of stable community that will emerge in an area is predicted by climate. This predicted ecosystem is called the climax community. When a climax community is extensive and well developed, it is called a biome. For example, at the Indiana dunes, the climax community is a temperate forest. This is because of the mean annual precipitation levels and mean annual temperatures that are common in the area of the Indiana dunes. Figure 14.10 shows the succession along a dune profile.

Since Britain has a similar mean annual precipitation and mean annual temperature to the Indiana dunes, the climax community of coastal sand dune succession is also a temperate forest. It is also formed in a similar fashion. Primary succession occurs along the coast as lyme grass and marram grass, which are the pioneer species, bind the sand in place. The youngest dune with these species is always closest to the shore. The woodland climax community is always the furthest from the shore, just as it is in the USA (see Figure 14.10).

NATURE OF SCIENCE

Theories of succession are now models being used by ecologists trying to restore the climax community to some natural areas that have been destroyed by human development. Restoration managers can manipulate the mechanisms of succession to achieve climax conditions more rapidly.

Sand dunes in the Outer Hebrides, UK

Marram grass

Analysis of data showing primary succession

In order to study primary succession, one group of researchers built a sand dike to model what would happen during 28 years of primary succession on a sand dune. See Figure 14.16.

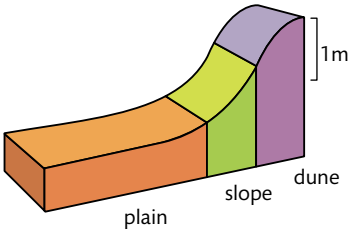


Figure 14.16 Sand dike study model showing plains, slope, and dune. Adapted from Olff et al. 1993, Fig. 1

The dike consisted of plains, slope, and dune. Over 28 years data were collected that are shown in these graphs.

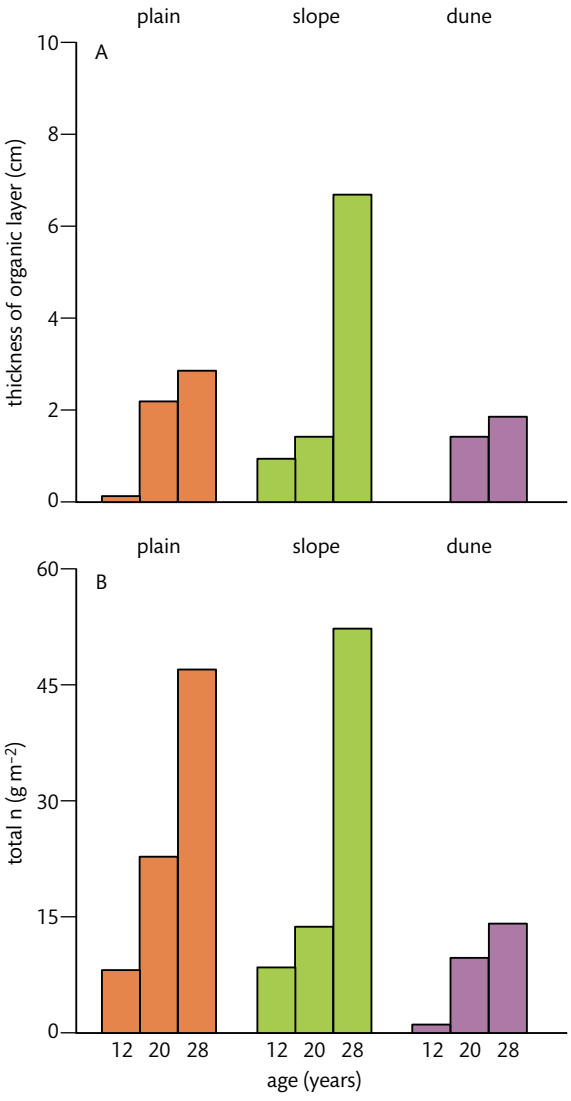


Figure 14.17 Primary succession as seen in the plain, slope, and dune dike.
A: Growth of the thickness over 28 years on the sand dike model.
B: Total amount of nitrogen in the organic layers over 28 years in three different areas on the sand dike model.
Olff et al. 1993, Fig. 10

CHALLENGE YOURSELF

11 Using Figure 14.17, compare and contrast the thickness of the organic layer of each area over time.

12 Using Figure 14.17, describe the difference in total nitrogen among the three areas.

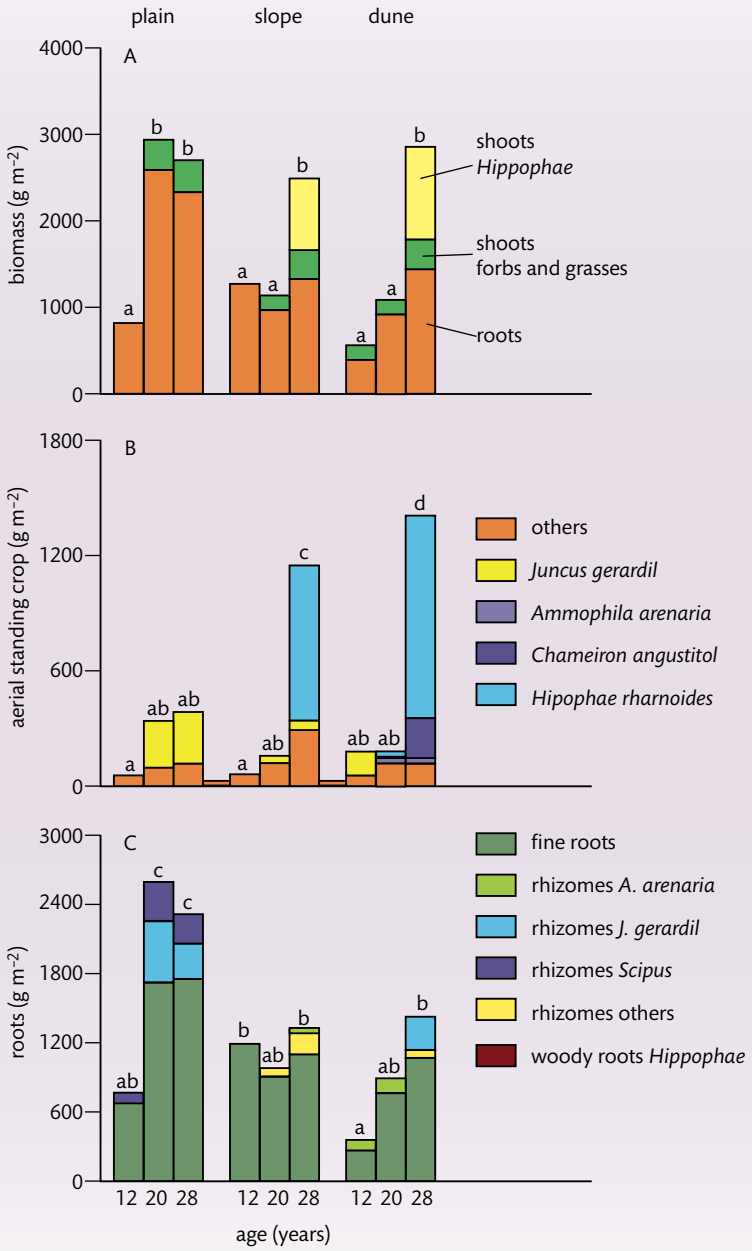


Figure 14.18 Reconstruction of total biomass **(A)**, above-ground biomass **(B)**, and below-ground biomass **(C)** of different plant species in Plain, Slope and Dune at three stages of primary succession. Totals with the same letter within each subfigure were not significantly different.
Olff et al. 1993, Fig. 6

13 Using Figure 14.18, describe the changes in biomass over the 28 years.

14 Using Figure 14.18, compare the changes in aerial standing crop for the slope and the dune.

15 Using Figure 14.18, which area had the largest increase in root mass? What environmental factor could have caused this?

Biosphere and biomes

If you view the surface of the Earth in a satellite picture, you can see large swathes of land covered with trees, other areas covered with ice, and other areas with nothing that can be seen. The living part of the Earth that you can see is called the biosphere. The

biosphere comprises all the parts of the Earth where organisms live. Some organisms live in the Earth's crust and some live in the atmosphere. Anywhere that organisms live is considered to be part of the biosphere.

Biomes are divisions of the biosphere. Each biome is a part of the biosphere and is defined by its vegetation and community structure.

Distribution of biomes

Biomes occur because of global weather patterns and topography (see Figure 14.19). Certain species are found in one type of biome and not in others.

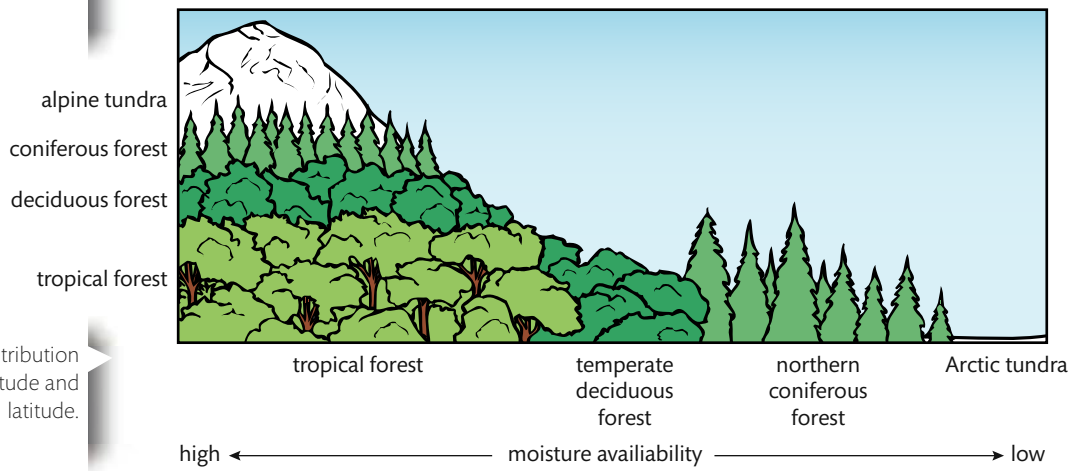


Figure 14.19 The distribution of some biomes by altitude and latitude.

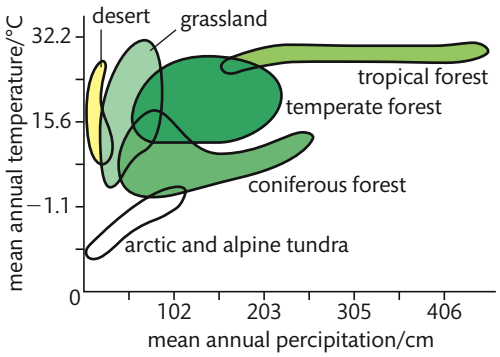


Figure 14.20 A climograph.

A climograph plots the temperature and rainfall in a particular region. In Figure 14.20, you can see that the mean annual precipitation (rainfall) is similar for a coniferous forest and a temperate forest, but the temperature is different. The mean annual temperature is colder for the coniferous forest. Compare the mean annual precipitation for grasslands and tropical forests. You can see that precipitation in the tropical forest is much higher. Rainfall and temperature affect the distribution of biomes.

The following combinations of temperature, rainfall, and elevation determine the biomes in North America.

Tundra

High elevations with low temperatures and low precipitation are the conditions that result in tundra. Plants and animals that live in the tundra are adapted to a cold and dry environment.

Coniferous forest

High elevations with less cold temperatures and slightly more rainfall are the conditions that result in coniferous forest. Because the ground freezes during some months of the year, coniferous (cone-bearing) trees are well adapted for conserving water when it is frozen. Animals have heavy coats of fur in the winter and lose some of the fur in the summer.

Temperate forest

At lower elevations, where temperatures are warmer and more water is available, the conditions produce temperate forest. Plants and animals in these forests must be adapted for a wide range of conditions: warm in the summer with lots of water, and

cool in the winter when water may be unavailable because it is frozen. Many trees in this forest will lose their leaves in the winter to reduce water loss.

Desert

At low elevations with warm temperatures and little precipitation, the conditions produce desert. Desert animals and plants have very specific adaptations that enable them to survive in this extremely hot and dry biome. A desert kangaroo rat has a specialized kidney for recycling water in its body. Cacti have spines instead of wide leaves to reduce water loss.

Tropical forest

At low elevations with warm temperatures and very high moisture, the conditions result in tropical forest. This forest is extremely productive, with high primary productivity as a result of the combination of high temperatures and high rainfall.

Table 14.7 Characteristics of the seven major biomes

Biome	Temperature	Moisture	Characteristics of vegetation
Desert	Mostly very hot with soil temperatures above 60°C (140°F) in the daytime	Low precipitation: less than 30 cm per year	Cacti and shrubs with water storage tissues, thick cuticles and other adaptations to reduce water loss
Grassland	Cold temperatures in winter and hot in summer	Seasonal drought is common with occasional fires, medium amount of moisture	Prairie grasses that hold the soil with their long roots; occasional fire prevents trees and shrubs from invading the grasslands
Shrubland (chaparral, matorral, maquis and garigue, dry heatherlands, fynbos)	Mild temperatures in winter and long, hot summers	Rainy winters and dry summers	Dry woody shrubs are killed by periodic fires. Shrubs store food in fire-resistant roots. They re-grow quickly and produce seed that germinates only after a fire
Temperate deciduous forest	Very hot in summer and very cold in winter	High rainfall spread evenly over the year. In winter, water may freeze for a short time	Deciduous trees like oak, hickory, and maple dominate the forest. In warmer seasons, a wide range of herbaceous plants grow and flower on the forest floor
Tropical rainforest	Very warm	Very high precipitation of more than 250 cm yr^{-1}	Plant diversity is high. A canopy of trees is the top layer. Next is a layer of shrubs. The ground layer is herbaceous plants and ferns. Large trees have vines climbing on them. Trees have orchids and bromeliads tucked in their branches
Tundra	Very cold; in summer, the upper layer of soil thaws but the lower layers remain frozen: this is permafrost	Little precipitation	Low-growing plants like lichen and mosses and a few grasses and shrubs. Permafrost prevents the roots from growing deeply. Continuous daylight in summer allows some plant growth and reproduction
Coniferous forest (taiga)	Slightly warmer than the tundra	Small amount of precipitation but wet due to lack of evaporation	Cone-bearing trees such as pine, spruce, fir and hemlock

Schmitt et al. 2011

CHALLENGE YOURSELF

Look at Figure 14.21.

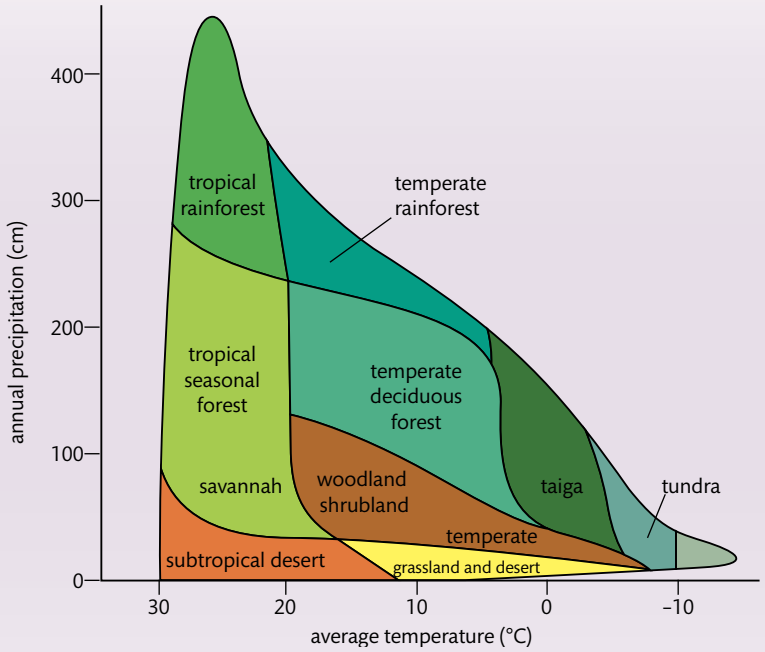


Figure 14.21 A climograph. This type of graph shows the relationship between temperature, rainfall, and ecosystem type. www.marietta.edu/-biol/biomes/desert.htm

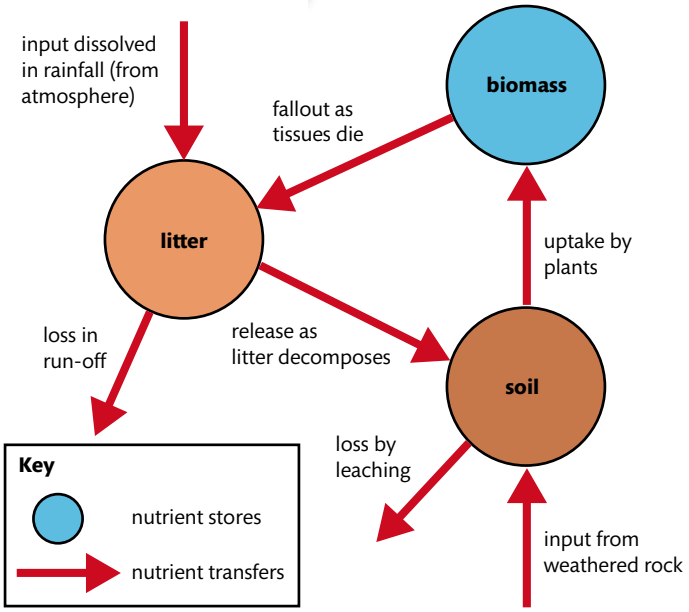
16 What is the average temperature range of a subtropical desert?

17 What is the highest amount of precipitation for a subtropical desert?

18 Compare the mean annual temperature for grasslands and tropical forests.

19 Compare and contrast the mean annual temperature for temperate deciduous forest and temperate rainforest.

Figure 14.22 A model of the mineral nutrient cycle.



Gersmehl diagrams

Another way of describing energy flows and nutrient recycling is to use Gersmehl diagrams of different biomes. These diagrams are a common method of demonstrating the cycling of nutrients within the main 'stores' of an ecosystem. As you will notice from the diagrams, the main stores of nutrients are soil, biomass (plants), and litter. Arrows of varying thickness represent nutrient transfer. Circles of varying size represent the size of the stores. Included in the diagrams are the following:

- input, such as of nitrogen, carbon, and minerals from weathered rock
- output, such as loses of nutrients by leaching and run-off
- flows, such as of leaf and needle fall from biomass to litter, and uptake of nutrients from the soil by plants.

Figure 14.22 shows a generalized model of a Gersmehl diagram.

Worked example

We will now look at a Gersmehl diagram for a specific biome, the tropical rainforest. First read this information about a rainforest, as it will help you make the diagram.

- Biomass is the main store of nutrients because the tropical rainforest has tall, dense vegetation with many layers and multiple species.
- Precipitation: rainfall is high all year.
- Litter has a very small store of nutrients because of the high rate of decomposition.
- Soil has a very small store of nutrients because of leaching and low soil fertility.
- Weathering (W) is rapid because of high heat and humidity.
- Leaching (Le) is high because of the high rainfall.
- Runoff (R) is high due to such large amounts of rain, that the soil cannot absorb it all.

Try to draw your own Gersmehl diagram before you look at Figure 14.23. Make sure to make the circles different sizes and use arrows of different thicknesses.

Solution

Now compare your diagram with the actual diagram.

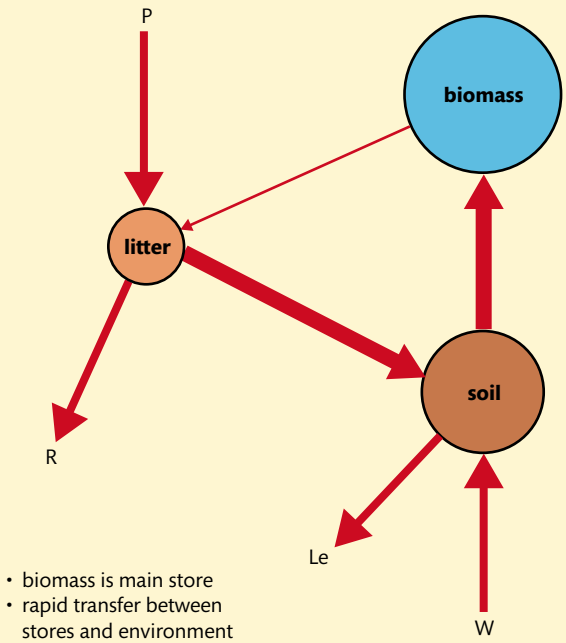


Figure 14.23 Gersmehl diagram of a tropical rainforest.

Gersmehl diagrams are models that predict and explain the natural world.

TOK

Worked example

We will now construct another Gersmehl diagram. This time the biome is taiga. Here are the specifics for taiga.

- Litter is the largest store of nutrients because of the low rate of decomposition as a result of low temperatures.
- Run-off is high. The ground is still frozen when the snow is melting.
- Biomass is relatively low because conifers have only one layer of needles and there is no undergrowth.
- Transfer from biomass to litter is high because of the constant supply of needles falling from coniferous trees.
- Soil stores are very small. Poor soil is formed from glacial deposits and so there is low soil fertility.
- Weathering of rocks is slow because of the cold.

Have a go at drawing your own diagram before looking at Figure 14.24; see if you are more accurate than you were with your first diagram.

Solution

Figure 14.24 shows the actual diagram for taiga.

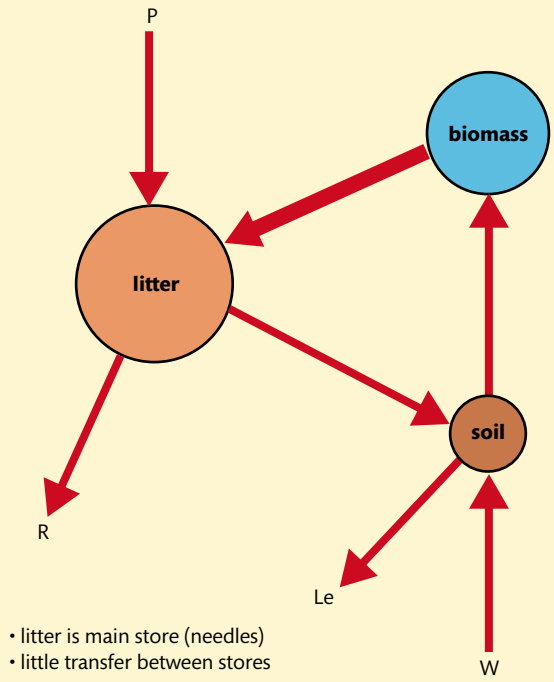


Figure 14.24 Gersmehl diagram of a boreal forest (taiga).

CHALLENGE YOURSELF

20 Draw a Gersmehl diagram for a desert biome. Here are the specifics you need:

- soil is rich in nutrients because there is little rain is available to wash them away
- biomass is small because of the extreme heat and lack of water
- litter or topsoil is practically non-existent because it is eroded by the wind
- run-off is high because there is no litter to hold on to the water
- loss as a result of leaching is low.

An example of humans interfering with nutrient cycling

In a study published in 2011 in the journal *Ecological Applications*, researchers documented how the collapse of marine fisheries as a result of overfishing and habitat loss has affected nutrient recycling in the marine environment.

Fish have a functional role in ecosystems. Through consumption and assimilation, fish recycle nutrients, especially nitrogen and phosphates, into forms that can be taken up by microorganisms and plants. The role of fish as nutrient recyclers is critical.

Eighty per cent of the nutrients that are used by primary producers are supplied by fish. Removal of fish tissues by marine fisheries in areas where nitrogen is low has affected primary production by plants. This has a negative effect on the herbivores in that community. In this study, estimates of nitrogen excretions rates for grey snapper in the Bahamas were 456% higher in unfished areas compared with fished areas. The excretion rates of phosphates were 451% higher in unfished areas compared with fished areas. The concern of these authors is that the sea grass beds that are the key habitat for young fish may be affected by this lack of recycled nutrients. Loss of primary production in the sea grass beds could cause the loss of even more fish.

A closed ecosystem

Most ecosystems are open. In a forest ecosystem, light enters and is trapped by plants. Herbivores eat the plants and their faeces fertilize the soil. After a fire, the soil may blow away to another ecosystem. Minerals may be leached by water after rain and be carried down the river to a new ecosystem.

Closed systems exchange energy but not matter. No natural system on Earth is considered to be a closed system, but the entire planet can be considered 'almost' closed. Large amounts of light energy enter the Earth and eventually return to space as heat, but matter is not exchanged.

Biosphere 2 Rainforest building.

Some experimentation has been done with artificial closed systems. A closed ecological system (CES) could be a space station. A space station does not rely on exchange of matter with its surroundings. In a closed ecosystem, waste products made by a species must be used by at least one other species. Waste products such as urine, faeces, and carbon dioxide must be converted into oxygen, food, and water. This involves at least one autotroph (green plant), which can use the waste products to make food as long as sunlight is available. Energy can be exchanged, but not matter.

An example of a large CES is Biosphere 2. Biosphere 2 is a large research facility, the size of two football fields, owned by the University of Arizona, USA. The research done here demonstrates the conditions that can affect a closed system. This facility has its own farm under a glass dome, and experiments are carried out with week-long periods of full closure, where humans live in the closed environment.

TOK

The old paradigm of diversity in an ecosystem stated that the most diversity is found in the oldest ecosystem. The intermediate disturbance hypothesis changed the way that ecologists think about how disturbances affect ecosystems. This hypothesis predicts that intermediate levels of disturbance promote higher levels of diversity.

Disturbances influence the structure and rate of change in an ecosystem

A disturbance is a new environmental condition that affects the structure and rate of change in an ecosystem. Examples of disturbances can be natural (for example fire, flood, wind, and insect invasion) or caused by humans (for example the clearing of a forest, building a road, ploughing a field, or clearing a natural area to build a housing development.)

In 1975, Joseph Connell proposed a new idea. His theory stated that disturbance is a common phenomenon that can actually have a beneficial effect on species diversity in a community. For example, fire in a forest affects the structure of the forest. By burning down the trees that shade the forest floor, the structure of the community is then changed, many more shade-intolerant plants can grow quickly, so the rate of change is affected.

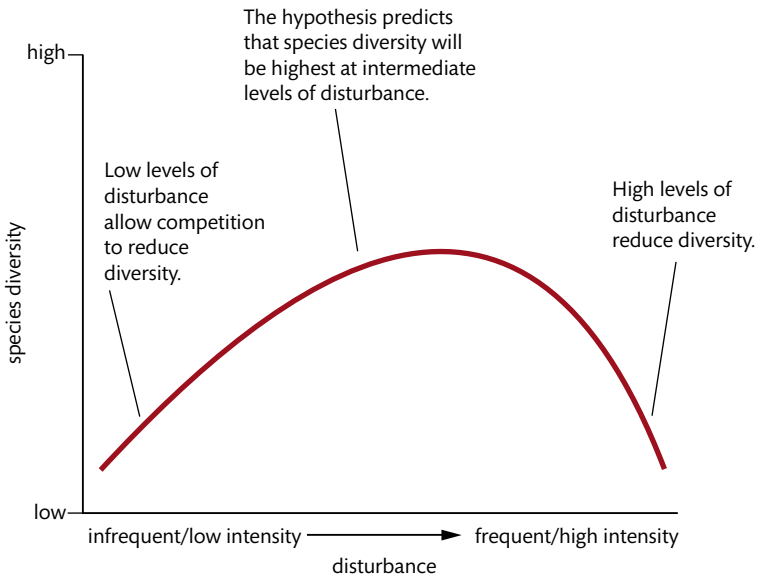


Figure 14.25 Graph of the disturbance hypothesis. Disturbance is a common phenomenon which may have a beneficial effect on species diversity in a community. Molles, Jr. 2010, Fig. 16.18

Look at Figure 14.25 and the points below.

- High levels of disturbance (e.g. constant mowing) reduce diversity. The community will only consist of the few species that can complete their life cycle between disturbances.
- Low (infrequent) disturbances will cause a decline in diversity because the species that are the best competitors will dominate.
- Intermediate levels of disturbances, such as a fire every few years, are the most effective at maintaining diversity. There is enough time between disturbances for a number of species to colonize an area. It can also slow the growth of dominant species.

Exercises

4 Describe a method of representing the cycling of nutrients in an ecosystem.

5 Explain how the Earth is a closed ecosystem.

6 Compare and contrast two energy pyramids from two different ecosystems.

C.3 Impact of humans on ecosystems

Understandings:

- Introduced alien species can escape into local ecosystems and become invasive.
- Competitive exclusion and the absence of predators can lead to reduction in the numbers of endemic species when alien species become invasive.
- Pollutants become concentrated in the tissues of organisms at higher trophic levels by biomagnification.
- Macroplastic and microplastic debris has accumulated in marine environments.

Applications and skills:

- Application: Study of the introduction of cane toads in Australia and one other local example of the introduction of an alien species.
- Application: Discussion of the trade-off between control of the malarial parasite and DDT pollution.
- Application: Case study of the impact of marine plastic debris on Laysan albatrosses and one other named species.
- Skills: Analysis of data illustrating the causes and consequences of biomagnification.
- Skills: Evaluation of eradication programmes and biological control as measures to reduce the impact of alien species.

NATURE OF SCIENCE

Assessing risks and benefits associated with scientific research: the use of biological control has associated risk and requires verification by tightly controlled experiments before it is approved.

Biological control: risks and benefits

Biological control is the idea of using a natural predator to control unwanted or invasive species. There are powerful arguments for using biological control. One argument is that biological control is an environmentally friendly alternative to chemical control. In fact a report by the National Academy of Sciences in 1987 argued that biological control should be the primary pest control method in the USA. When an invasive species is affecting an entire community, even cautious observers would agree that biological control should be considered. However, there is always a risk when introducing a new organism into an ecosystem. Unexpected consequences may occur even though rigorous testing is carried out beforehand. Scientists look at risk–benefit analyses and make decisions based on those analyses.

Introduced alien species can become invasive

One of the classic examples of biological control 'gone wrong' is the introduction of cane toads, *Rhinella marina*, into Australia in the 1930s. The cane toad was imported from Hawaii and released in Queensland to control the beetle pests of sugar cane.

The larvae of the beetle pests of sugar cane eat the roots of the cane and the plants die. Cane growers were interested in controlling the beetle pests because sugar cane crops are a major income producer in Australia. Entomologists researched many solutions to the sugar cane pest problem, such as chemical insecticides, soil fumigation methods, and physical removal. After 25 years, none showed much promise in field trials.

In 1935 one entomologist was sure he had found the solution. In Hawaii, the cane toad was being used to control beetle infestations of sugar cane crops. This idea was quickly accepted in Australia. In June 1935, 2400 toads were released in an area of Queensland. Other entomologists had argued against this quick release. Risk assessments of the potential harm of introducing these toads had not been done. Unfortunately, the

outcome has been that this poisonous toad has multiplied very rapidly, currently the population has grown to more than 200 million, yet the beetles that the cane toad was brought in to eradicate have not been affected. It seems that the beetle pests were not affected because in Australia, compared with Hawaii, the toads have found other food sources, such as smaller beetles and moths. By 1950 the cane toad was recognized as a pest in the Vermin Act, but it wasn't until the 1980s that the cane toad was recognized as a national issue. Lessons learnt from the cane toad debacle are probably one of the reasons why Australia today has very strict quarantine laws and tough risk-assessment procedures.

Australia is the only nation with a specific law for classical biological control, the Australian Biological Control Act of 1984. Most state laws in the USA encourage the use of biological control, but adequate supervision is lacking. There is an international organization, the International Organization for Biological Control (IOBC), working to promote environmentally safe practices around the world.

- **Cane toads were brought to Australia to control the sugar cane beetles biologically.**
- **Cane toads have no natural predators in Australia because they are toxic to Australian crocodiles and large lizards.**
- **Cane toads reproduce rapidly.**
- **Cane toads have become more of a pest in Australia than the beetles they were meant to eat.**
- **Cane toads have found plenty of other species to eat in Australia, so they ignore the target beetle pests.**
- **The risk assessment carried out before the introduction of cane toads to Australia was not adequate.**

Competitive exclusion can affect endemic species

The principle of competitive exclusion states that no two species can occupy the same niche. When two species have a similar need for the same resources, such as food, one will be excluded. In Australia, when the cane toads attain high population densities, they consume a large number of invertebrates. Individual cane toads are thought to consume 200 beetles, ants, and termites per night. A report from the National Cane Toad Task Force of Australia in June 2005 showed that a decline in some small reptile species has coincided with the increase in the cane toad population. Competition for food is inferred as the reason for the population declines. A burrowing frog, *Limnodynastes ornatus*, shows no survival of tadpoles in ponds where cane toad tadpoles are present. Direct predation on tadpoles is not significant, which is why competition for food is inferred. Small skinks (blue-tongued lizards) begin to disappear once the cane toad arrives. Both species are insectivores, and it is hypothesized that the skinks are outcompeted by the cane toad for insects because of the voracious appetite of the toad. These examples are evidence that endemic species are being outcompeted by the cane toads. Many more studies are being carried out by scientists in Australia to discover what exactly the results are of this attempt at biological control.

The eastern blue-tongued skink.

The cane toad.

Absence of predators can affect endemic species

Cane toads are extremely poisonous. Behind the ears of cane toads are glands that contain a toxic substance. Predator species in Australia are seriously affected by eating cane toads. Seventy-five species of turtles are at risk because they can eat toads large enough to kill them with their toxin; 90% of large lizards die after eating cane toads. Other evidence has shown an impact on snakes and crocodiles that have eaten cane toads. The absence of a successful predator of cane toads is a significant factor in the population explosion of cane toads in Australia.

New research on cane toads in northern Australia has suggested that a good way to control the cane toad invasion is to use parasites that are specific to cane toads.

Kudzu: an introduced alien species

What we do to the environment today may have unforeseen consequences for future generations. Kudzu was introduced from Japan to the USA in 1876 at the Philadelphia Centennial Exposition as an ornamental plant. In the 1930s it was promoted by the Soil Conservation Service of the US government as a fast-growing plant that could solve the problem of soil erosion. From 1935 to 1950 it was planted by the Civilian Conservation Corp sponsored by the federal government. Then, in 1953, it was recognized by the US Department of Agriculture as a pest weed.

Currently, kudzu is common throughout the southeastern states of the USA. It is often called 'the plant that ate the South'. Here is the reason why: kudzu grows rapidly, as much as 20 m per season. Thirty stems can emerge from one root. It grows both horizontally and vertically. Kudzu spreads by runners that can make roots and produce more plants. Kudzu grows well in many conditions, although prolonged freezing will kill it. The thick growth crushes other plants as it covers them. Its weight breaks tree branches. In the USA, the effects of kudzu cost $500 million annually.

TOK

Will our knowledge of the damage that biological control can do, if not monitored well, change the methods we use in the future?

Kudzu, the plant that ate the South.

Biomagnification

Biomagnification is a process by which chemical substances become more concentrated at each trophic level.

When chemicals are released into the environment they may be taken up by plants. The plants may not be affected by the small amount of a chemical that they absorb or have on their surface. But when large amounts of the affected plants are eaten by a primary consumer, the amount of chemical the consumer takes in is much greater. Similarly, if numbers of the primary consumer are eaten by a secondary consumer, the amount of chemical taken in by the secondary consumer is magnified even more. Chemicals that are biomagnified in this manner are fat soluble. After ingestion, they are stored in the fatty tissue of the consumer. When the consumer is caught and eaten, the fat is digested and the chemical moves to the fatty tissue of the secondary consumer.

Causes of biomagnification

Some toxic chemicals have been put deliberately into the environment to kill insect pests. One of these pesticides is dichlorodiphenyltrichloroethane (DDT), which has been used to control mosquitoes and other insect pests. At the time it was first used, it was not known that DDT does not break down and can persist for decades in the environment. DDT was commonly sprayed on plants and eventually entered water supplies. There it was absorbed by microscopic organisms. These microorganisms were eaten by small fish, and the small fish were eaten by larger fish. DDT built up in the fatty tissues of the fish. When these fish were eaten by birds, the magnification of DDT was even greater (see Figure 14.26).

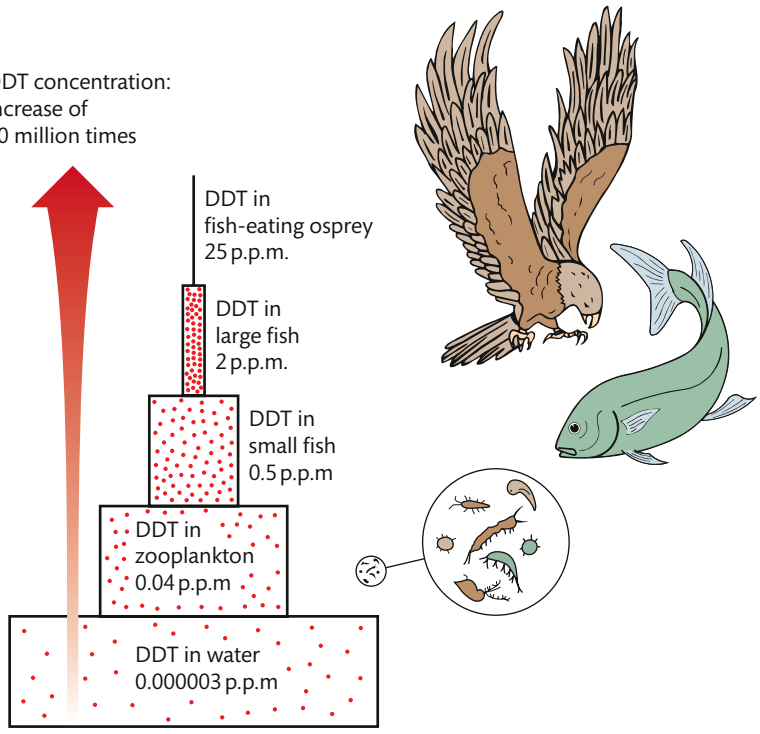
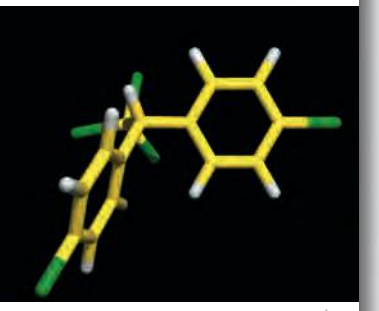


Figure 14.26 Biomagnification of DDT.

The proposal to commit to a deadline for a worldwide ban on the pesticide DDT by 2020 was rejected at the Sixth Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants. India, the largest producer of DDT, strongly opposed the proposal. India is the only country still manufacturing DDT.

A feeding mosquito.



DDT.

Consequences of biomagnification

The first sign of the problem with DDT was a decline in the number of predator birds. Studies showed that the eggs of these birds were easily cracked. In fact, the weight of the mother sitting on the eggs cracked them. It was finally discovered that DDT was building up in the tissue of the birds and interfering with the calcium needed for the shells to be hard. DDT was banned in the USA in 1971. The bird population has begun to recover following the ban. DDT was originally banned because of its effects on birds. However, It also affects humans who consume agricultural products and eat fish containing accumulations of DDT. Because DDT is stored in fat, levels of DDT in breast milk are often six times higher in a mother than in her blood.

The trade-off between DDT pollution and malarial parasite control

What are the challenges that must be overcome as we face decisions over DDT pollution and malarial parasite control? Malaria is the most deadly vector-borne disease in the world. It kills more than 1 million people per year. In the past 25 years, there has seen a dramatic rise in cases of malaria, despite the use of insecticide-treated bed nets (ITNs), indoor resident spraying (IRS), and artemesin combination therapy (ACTs, an antimalarial drug). How can we overcome this challenge? Should the trade-off be the environment or human health?

DDT pollution

We have just looked at some of the problems caused by biological magnification of DDT. DDT is a persistent organic pollutant (POP). These pay-offs have followed the

ban on DDT in the USA. Peregrine falcons have come off the endangered species list; bald eagles will soon follow. DDT levels in human blood samples have declined sharply. DDT has disappeared from the breast milk of nursing mothers.

Control of malaria for large human populations

The difficulties of malaria control for some nations are significant. When IRS is used in houses, DDT can be found in the breast milk of nursing mothers. Without IRS, hundreds of mosquitoes can enter a house, compared with no mosquitos entering a house that has been sprayed. In Africa and Indonesia, malaria is more of a problem than human immunodeficiency virus (HIV). Many health officials would like to use DDT to ease the suffering of human populations, but donor governments refuse to allow DDT spraying. A documentary movie shows the effect of refusing to spray DDT for human populations in underdeveloped countries; *3 Billion and Counting*, tells the story of the devastation of malaria on large populations of people.

Macroplastics in the marine environment

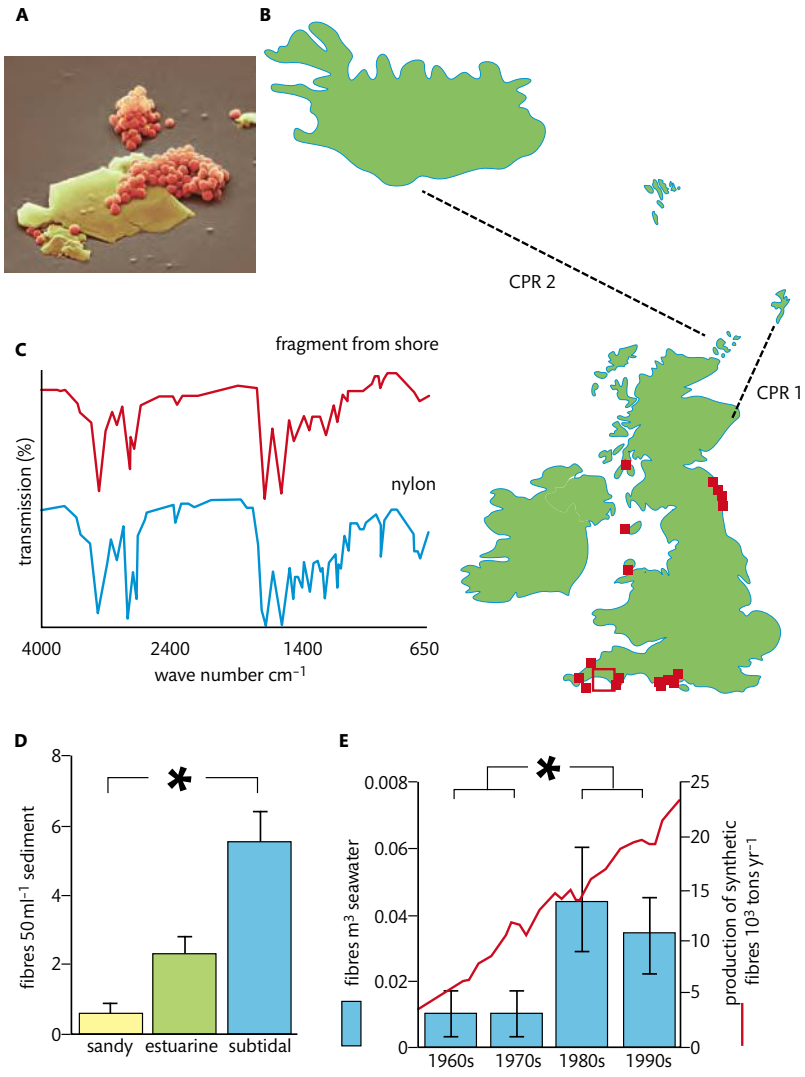


Figure 14.27 **(A)** Facial scrub particles shown in an electron micrograph are microplastics polluting the ocean. **(B)** Sampling locations in North-East Atlantic, showing Routes sampled by Continuous Plankton Recorder (CPR 1 and 2) since 1960 and used to assess the abundance of microplastics in the water column. Red squares indicate the abundance of microplastics. **(C)** Example showing how FT-IR spectroscopy was used to identify fragments from the environment. Here an unknown fragment is identified as nylon. **(D)** There were significant differences in abundance of microplastics between sandy beaches and subtidal habitats , but abundance was consistent among sites within habitat type. **(E)** Accumulation of microscopic plastic in CPR samples revealed a significant increase over time when comparing the 1960s and 1970s to the 1980s and 1990s. Approximate figures for global production of synthetic fibres (red line) overlain for comparison. Microplastics were also less abundant along the oceanic route CPR2 than CPR 1. To read the article where this figure is adapted from ('Lost at sea: Where is all the plastic?'), go to the hotlinks site, and click on Chapter 14: Section C.3. http://www.kimointernational.org/MicroPlasticResearch.aspx

The Marine Conservancy has published the estimated decomposition rates of most plastic debris found on coasts.

- Polystyrene cups: 50 years.
- Plastic drinks containers: 400 years.
- Disposable nappies: 450 years.
- Plastic bottles: 450 years.

A Laysan albatross with regurgitated waste.

A sea lion caught in a fishing net.

Macroplastics are pieces of plastic bigger than 5 mm. The accumulation of macroplastics is very high adjacent to urban areas in the northern hemisphere. Macroplastic items such as plastic bottles, bags, nets, fishing lines, and many items of rubbish can pose a serious threat to marine wildlife.

- Marine mammals and turtles can ingest plastic bags and bottles, which then interfere with their digestive system.
- Drift nets can entangle birds, fish, and mammals.
- PCBs and other contaminants may be concentrated in pieces of plastic pieces that are ingested by birds, fish, and mammals.

The Laysan albatross

Seabirds can mistake plastic floating on the surface of water as food and ingest it. Because most adult birds regurgitate food to feed their young, the plastic is passed on to the chicks. One study discovered that 98% of Laysan albatross, *Phoebastria immutabilis*, chicks had been fed beads, toys, golf tees, buttons, and many other plastic items by their parents. It seems that the albatross prefers pink items. It is hypothesized that pink items are the same colour as, and are mistaken for, shrimps. These items can obstruct a chick's digestive system, leading to starvation and death. One Laysan albatross chick had a piece of plastic 11 cm long embedded in its gut.

Microplastics in the marine environment

Microplastics are defined as plastic particles smaller than 5 mm. They are produced directly as abrasives and exfoliants, or indirectly as a consequence of the breakdown of larger plastic material. The amount and distribution of microplastic particles in the marine environment has increased steadily over the past 20 years because of the rise in the use of plastics by humans. It has been found that microplastics act like sponges and soak up toxic chemicals such as PCBs in the marine environment. Microplastics have a large surface area in relation to their size, so there is plenty of surface for the chemicals to stick to. The potential impacts of microplastics on the marine environment include:

- accumulation of more and more plastic debris, because these plastics do not break down
- ingestion of microplastic pieces by marine organisms, causing damage to their digestive systems
- absorption of components of the plastic, or chemicals adsorbed on the plastics, by the ingesting organism
- accumulation of those chemicals in the body of the ingesting organism.

Research is ongoing to determine whether microplastics themselves or the chemicals they contain are carried across trophic levels. For example, do PCBs or other POPs adsorbed onto plastic pieces build up in the bodies of fish and become magnified as the small fish are eaten by larger fish, etc.? Biomagnification of chemicals from plastics, either within the plastics or adsorbed onto the plastic pieces, may be occurring.

Impact of microplastic debris on lugworms

Lugworms, *Arenicola marina*, are called the earthworms of the sea. They are commonly found in the USA and Europe and are used as bait by fishermen. They feed on ocean floor sediments by striping the sediment of debris and organic matter. They can eat

sand particles and digest the microorganisms and nutrients on the surface of the sand particles. Because they are basically 'eating' what has fallen to the bottom of the sea, their health can tell us about the health of the oceans, making them indicator organisms. In one experiment, a California scientist, Mark Anthony Browne, found that, when lugworms eat microplastic pieces contaminated with common chemical pollutants, those pollutants are found in high concentrations in the lugworm's tissues. These high concentrations make the lugworms vulnerable to pathogens. They cause the worms to have less energy for churning up the bottom sediments, which is one of their most important functions in the ocean ecosystem. This is a key finding because the current policy in the USA considers microplastics to be non-hazardous.

A lugworm casting on a beach.

Mark Anthony Browne from the University of California at Santa Barbara has joined with UK scientists from Plymouth University to study lugworms as they feed on highly contaminated ocean sediments.

Biological control and eradication programmes to reduce the impact of alien species

Invasive alien species are recognized as a serious biological threat to the environment and to economic development. Without any natural predators, a plant or animal that has been moved out of its local ecosystem may multiply and threaten other species, agriculture, or public health. Many nations are grappling with the complex and costly problems caused by invasive alien species organisms, such as the black striped mussel and fire ants. The following examples describe some strategies that have been used to eradicate invasive alien species.

Water hyacinths are aquatic plants native to the Amazon basin, but are often considered an invasive species outside of their native range.

The black striped mussel, *Mytilopsis sallei*, was found to have invaded Cullen Bay in Darwin Harbor, Australia, in 1988. This mussel is capable of making a thick matt more than 10 cm thick and can foul anchors, pylons and buoys, storm water pipes, vessel hulls, and breakwaters. It is a very serious threat to tropical Australian waters. Regular surveys of Australian ports highlighted the discovery of the mussel within 6 months of its arrival. A rapid decision to eradicate it prevented the spread. The method of eradication was to close the gates to the marina where they were located and expose the entire area to copper sulfate and chlorine. These chemicals were poured into the water, killing the mussels and all other living organisms in the marina. The potential economic damage to the pearl industry from black mussels is $350 million a year, and damage to the prawn fishery catch is worth close to $120 million a year at today's values.

International Pellet Watch (IPW) was founded in 2005 by Dr Hideshige Takada of Tokoyo University. He has asked citizens across the globe to collect plastic pellets from the beaches they visit and send them to his laboratory. He analyses the POP content of the pellets and their global distribution. The results are sent back to donors by email and released on the web. So far pellet samples have been analysed from 200 locations and 40 countries. About 1000 pellets have been analysed, and POPs have been detected in every one of those 1000 samples.

Imported red fire ants on a wooden stick.

Fire ants, *Solenopsis invicta*, are notorious because of their painful, burning stings. The stings result in pustules, which continue to itch intensively. Fire ants attack humans as they walk across lawns and golf courses. Red fire ants also attack livestock grazing in the fields of Florida. Fire ants were brought to Florida from South America. Use of insecticides kills native ants as well as fire ants, but fire ants return much more quickly than the native ants. Insecticides are the primary control of fire ants today in Florida. The estimated cost of red fire ant control in Florida is $36 per household.

A biological control agent that is being tested against fire ants is a fly of the genus *Pseudacteon*. If the fly catches an ant, it lays its eggs inside the head of the fire ant. When the eggs hatch, the larvae eat through the head of the fire ant. *Pseudacteon* flies might work but they might not control the fire ants. They could also become a pest because they have no predators. In South America, the fire ant population is only one-fifth the size of that in southern USA. Scientists hypothesize that the 24 species of *Pseudacteon* flies native to South America keep the fire ant under control. In 2011, one species of *Pseudacteon* fly was introduced to control fire ants in Florida. It was found that it did not have a significant impact on the fire ant population after its release. The study concluded that multiple species of flies would be required in order to replicate the conditions of natural fire ant control seen in South America. Would Florida be inviting new pests to the sunshine state, in the hope of ridding themselves of an old pest?

The oriental fruit fly, *Bactrocera dorsalis*, was introduced into Okinawa Island in Japan in 1945. It damages fruit crops by laying eggs in the fruit: the larvae from the eggs eat the fruit. All Japanese territories were declared free of the oriental fruit fly in 1985, because of an 18-year eradication programme combining some insecticide use with sterile insect release (SIR). SIR entails raising large numbers of sterile male flies and releasing them to mate with wild females. The numbers of released sterile males are very high. In one study, 648 sterile males were released for every one wild male in the wild population. However, according to another study it is enough to release nine sterile males to one wild male. When the males are released and mate with wild females, the eggs from these females are not viable. After SIR release on one island, the percentage of infestation of host fruits decreased to zero in 3 months. The success of SIR and strict inspection of incoming produce that might contain this fruit fly means that Japan has been free of the oriental fruit fly for the past 20 years.

An adult female oriental fruit fly, *Bactrocera doralis*.

CHALLENGE YOURSELF

21 Evaluate the eradication programmes described above by yourself or with a friend.

Analysis of data illustrating the cause and effects of biomagnification

As we have learned previously, biomagnification is the build-up of heavy metals and POPs by successive trophic levels, resulting in higher concentrations in the predator organisms than in the prey. Bioaccumulation is the net accumulation over time of POPs or heavy metals (such as mercury, arsenic, and lead) within an organism from both biotic (other organisms) and abiotic (soil, water, etc.) sources.

Whenever a question in an IB exam asks you to 'evaluate' something, it means to state both the risks and the benefits. It does not mean give just your opinion. Creating a table is a good way of answering this type of question.

CHALLENGE YOURSELF

The data you will analyse were collected from streams in the USA, from the state of Missouri, in an area called the Ozarks. The heavy metal that is causing concern there is mercury. It is hypothesized that mercury is bioaccumulating in several fish that are commonly caught and eaten by the people in this area.

Mercury (Hg) is a pollutant from both natural and human sources. Mercury found in aquatic systems comes from leaf litter: leaves accumulate atmospheric mercury when alive and then fall into streams when dead. Wet areas are conducive to changing the inorganic mercury in the leaves into methyl mercury (MeHg), which is subject to bioaccumulation and biomagnification. MeHg is highly toxic to fish, wildlife, and humans. In the USA, nationwide fish consumption guidelines have been developed. For example, Missouri guidelines suggest not consuming a fillet of fish that is more than 0.3 $\mu g\ g^{-1}$ wet weight of the whole fish because of MeHg accumulation. Recreational fishermen in the Ozarks catch hogsuckers and smallmouth bass. The fatty tissues of both these fish typically exceed the amount of MeHg recommended as safe in Missouri.

Figure 14.28 shows the food web for the study you are about to analyse, and an explanation of the organisms that are involved in the biomagnification process.

TOK Can you use inductive reasoning to formulate a hypothesis about the effectiveness of biological control?

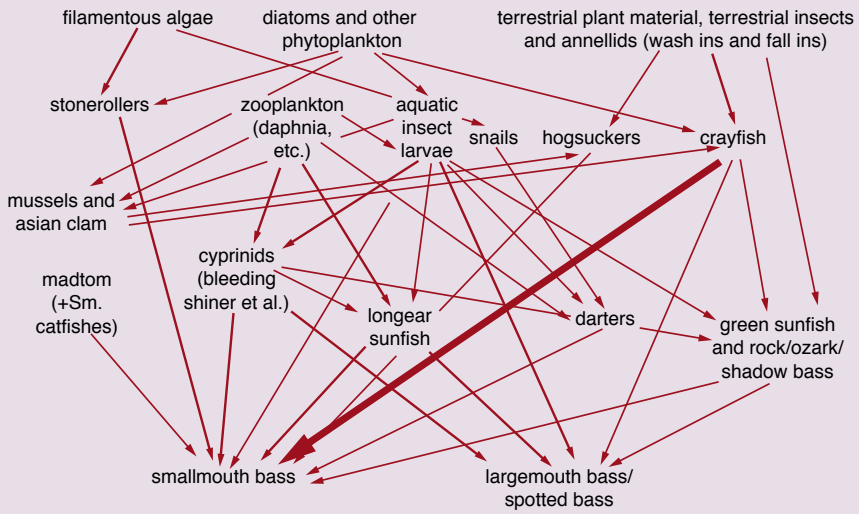


Figure 14.28 Simplified food web. Adapted from www.combat-fishing.com/ reproduced with permission

The organisms involved in this process are described below.

The Asian clam *Coribicula* is a filter-feeding mollusc that ingests fine particulate organic matter from leaf litter and algae.

Crayfish, *Cambarus*, are crustaceans that are omnivorous primary consumers; they eat leaf litter, and aquatic invertebrates such as clams and fish.

Hogsuckers, *Hypentelium nigricans*, are bottom-feeding fish that eat aquatic invertebrates such as crayfish, and organic matter from the stream bottom.

Smallmouth bass, *Micropterus dolomieu*, are fish that feed primarily on crayfish, along with other aquatic invertebrates and small fish.

Look at the map of sites (Figure 14.29) sampled by scientists from the University of Nebraska at Lincoln. Figure 14.29 and Table 14.7 tell you where the samples were taken and the name of the sites. You will need this information when you analyse the data from the following graphs.

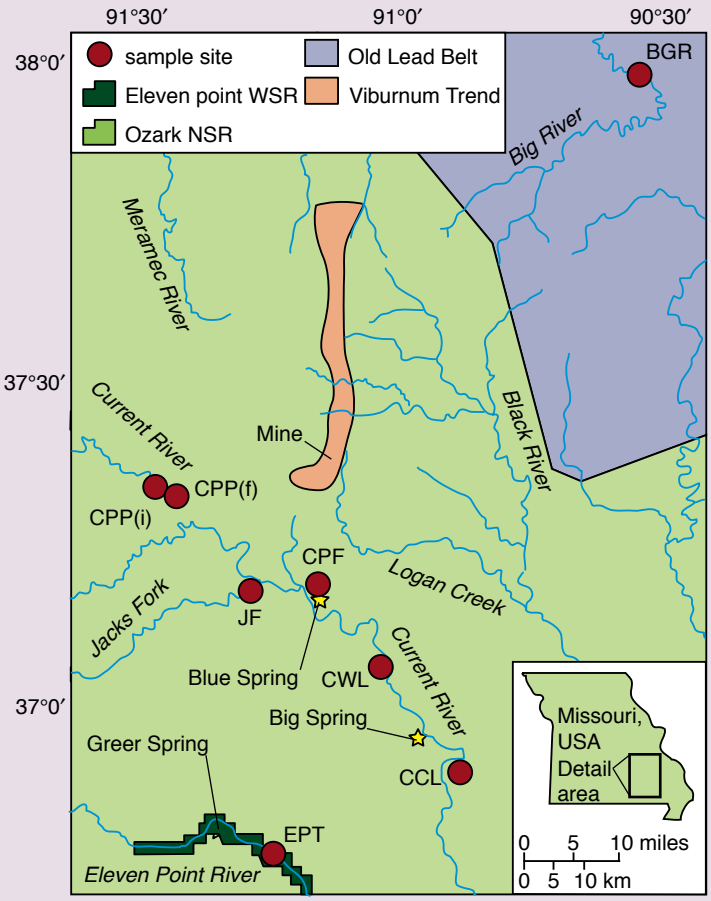


Figure 14.29 Map showing collection sites, lead-zinc mining area (Viburnum Trend and Old Lead Belt), and boundaries of the Eleven Point Wild and Scenic River and boundaries of the Ozark National Scenic Riverways. Schmitt et al. 2011, Fig.1

Table 14.7 The collection sites for the fish

Collection sites for fish (f) and invertebrate (i; crayfish and *Corbicula*).

Site	River	Location	Latitude, longitude [b]
$EPT_{i,f}$	Eleven Point	Turners Mill	36°45′56.7″N, 91°16′01.0″W
CPP_{i}	Current	Pulltite Landing	37°20′04.1″N, 91°28′33.8″W
CPP_{f}	Current	Presley Center	37°19′12.6″N, 91°26′14.6″W
$JF_{i,f}$	Jacks Fork	Shawnee Creek	37°10′21.3″N, 91°18′00.6″W
$CPF_{i,f}$	Current	Powdermill Ferry	37°10′48.0″N, 91°10′25.0″W
$CWL_{i,f}$	Current	Waymeyer Landing	37°03′15.1″N, 91°03′16.8″W
$CCL_{i,f}$	Current	Cataract Landing	36°53′22.2″N, 90°54′473″W
$BGR_{i,f}$	Big	St Francois State Park	37°57′23.7″N, 90°32′29.2″W

Schmitt et al. 2011, Tab. 1

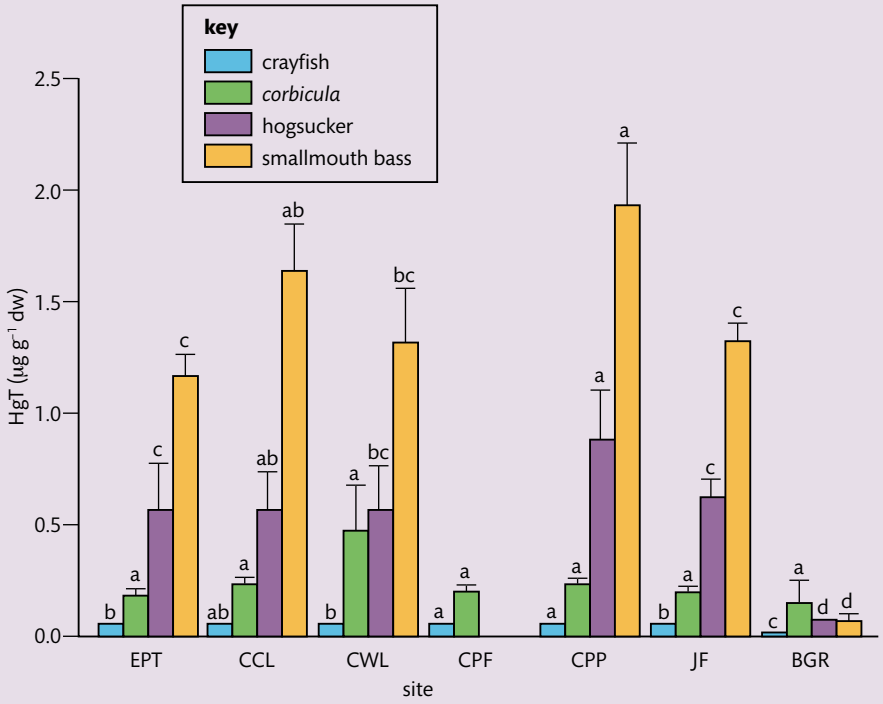


Figure 14.30 Total mercury (HgT) arithmetic site means (±standard errors) in *Corbicula*, crayfish, hogsuckers, and smallmouth bass. Within taxa, means sharing the same letter are not significantly different (ANOVA, $p<0.05$). See **Figure 14.29** and **Table 14.7** for site names and locations.
Figure 14.30: Schmitt et al. 2011, Fig. 2

Look at the graph Figure 14.30 of mercury build-up at these sites.

22 Distinguish between the concentration of HgT in crayfish and smallmouth bass at all the sites where both were collected.

23 Compare and contrast HgT at the two sites CPP and BGR.

24 Across all the sites, which organism had the highest concentration of HgT? Suggest a reason for this.

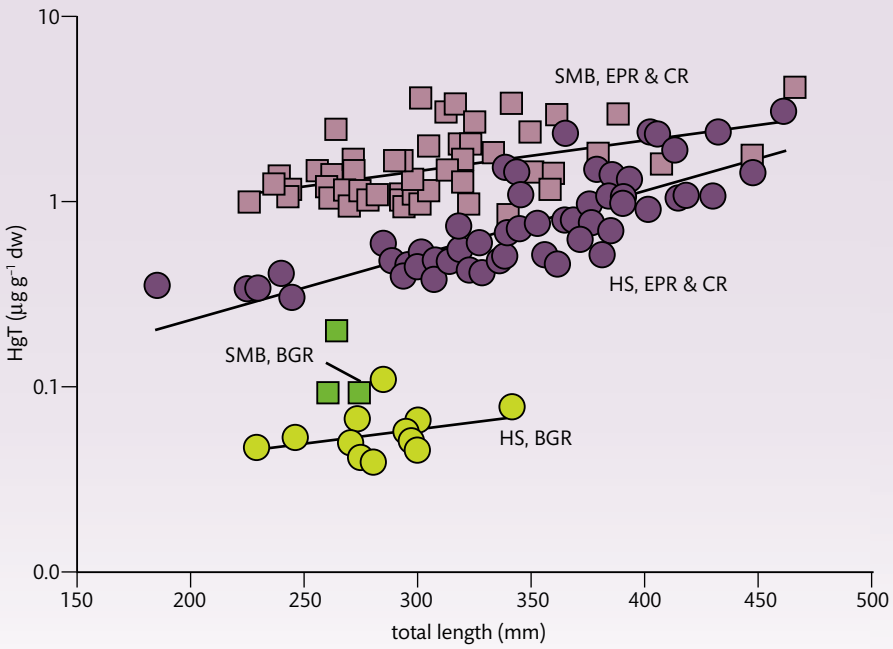


Figure 14.31 Total mercury (HgT; *y*) versus total length (*x*) in hogsuckers (HS) and smallmouth bass (SMB) from the Current River (CR), Eleven Point River (EPR), and Big River (BGR). Adapted from Schmitt et al. 2011, Fig. 3

25 Using Figure 14.31, was it length or site that contributed to HgT accumulation?

26 If you were an Ozark fisherman, at which site would you choose to fish? Explain your answer.

To learn more about the DDT controversy, go to the hotlinks site, search for the title or ISBN, and click on Chapter 14: Section C.3

Exercises

7 Explain how competitive exclusion can affect endemic species.

8 Describe the effect of microplastics on the marine ecosystem.

9 Discuss the trade-offs between DDT and control of malarial parasites.

NATURE OF SCIENCE

Scientists collaborate with other agencies: the preservation of species involves international cooperation through intergovernmental and non-governmental organizations.

C.4 Conservation of biodiversity

Understandings:

- An indicator species is an organism used to assess a specific environmental condition.
- Relative numbers of indicator species can be used to calculate the value of a biotic index.
- *In situ* conservation may require active management of nature reserves or national parks.
- *Ex situ* conservation is the preservation of species outside their natural habitats.
- Biogeographic factors affect species diversity.
- Richness and evenness are components of biodiversity.

Applications and skills:

- Application: Case study of the captive breeding and reintroduction of an endangered animal species.
- Application: Analysis of the impact of biogeographic factors on diversity limited to island size and edge effects.
- Skill: Analysis of the biodiversity of two local communities using Simpson's reciprocal index of diversity.

Guidance

- *The formula for Simpson's reciprocal index of diversity is:*

$$D = \frac{N(N-1)}{\Sigma\, n(n-1)}$$

D = diversity index, N = total number of organisms of all species found, and n = number of individuals of a particular species.

Indicator species and biotic indices

Do you remember reading stories of coal miners taking canaries into the mines? If the canary died, it indicated the presence of poisonous gas. In an ecosystem, some species are like those canaries. They are very sensitive to environmental change. They are called indicator species.

Some indicator species

A common indicator species is lichen. Lichens live on rocks and trees and are a reliable indicator of air quality. They are very sensitive to pollution in the atmosphere. Lichens are not usually found on trees in a city because the air is too polluted for them. Because lichens also retain metal in their tissues, they can show the presence of lead or mercury in the air.

Another group of indicator species are macroinvertebrates found in rivers and streams (see Figure 14.32). The presence or absence of these organisms can be used to judge the water quality.

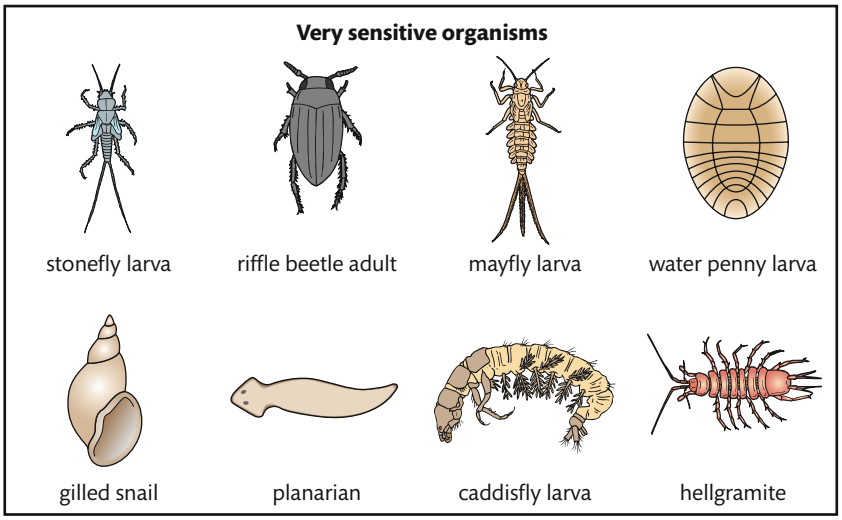
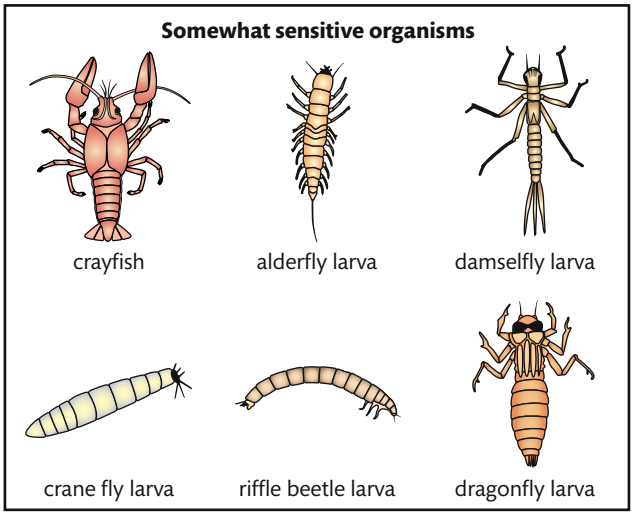
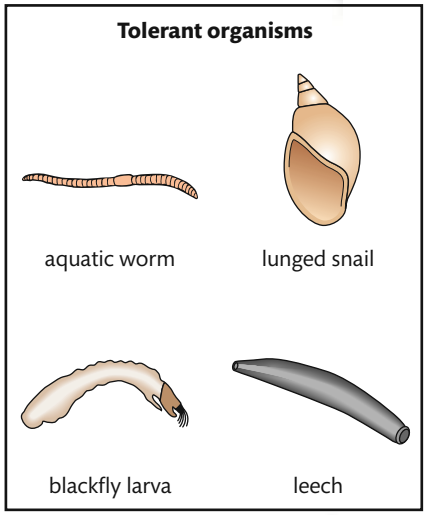


Figure 14.32 Some macroinvertebrates that are indicator species.





We are all interested in the quality of our rivers and streams. In the recent past, rivers and streams were often used as dumping grounds for toxic chemicals and unwanted materials. In Chicago, USA, around 1900, the river was a dumping ground for the waste products from the slaughter houses. All of the unwanted parts of the animals were thrown into the Chicago River. In fact, one branch of the river was named 'bubbly creek' because of all the fermentation that was taking place as the animal tissue decomposed in the water. Today, that river is a much cleaner place, with boats and canoes floating on it rather than waste. This change has come about because of our increased awareness that water and waterways are precious commodities to be treasured.

Freshwater indicator species have various levels of pollution tolerance. Organisms like leeches and aquatic worms are not very sensitive and can live in water with low oxygen levels and high amounts of organic matter. Organisms like the larvae of alderfly and damselfly are moderately sensitive, whereas the larvae of the mayfly and caddisfly are very sensitive to pollution. The very sensitive organisms must have high levels of oxygen and little organic matter in the water in order to survive. The cleaner the water, the higher the number of sensitive organisms present.

Stream study: Sample record and assessment

Stream _____ Site number _____

County or city _____ State _____

Collection date _____ Collectors _____

Weather conditions (last 3 days) _____

Average depth at site _____ Average width at site _____

Water temperature _____ °C _____ °F

Flow rate: ☐ High ☐ Normal ☐ Low

Appearance: ☐ Clear ☐ Cloudy ☐ Muddy

Macroinvertebrate count

Sensitive	Somewhat sensitive	Tolerant
☐ __ caddisfly larvae	☐ __ beetle larvae	☐ __ aquatic worms
☐ __ hellgramite	☐ __ clams	☐ __ blackfly larvae
☐ __ mayfly larvae	☐ __ crane fly larvae	☐ __ leeches
☐ __ gilled snails	☐ __ crayfish	☐ __ midge larvae
☐ __ riffle beetle adult	☐ __ damselfly larvae	☐ __ lunged snails
☐ __ stonefly larvae	☐ __ dragonfly larvae	
☐ __ water penny larvae	☐ __ scuds	
	☐ __ sowbugs	
	☐ __ fishfly larvae	
	☐ __ alderfly larvae	
	☐ __ watersnipe larvae	
boxes checked × 3 = _____ index value	boxes checked × 2 = _____ index value	boxes checked × 1 = _____ index value

Water quality rating ☐ Excellent (>22) ☐ Fair (11–16)

Total index count _____ ☐ Good (17–22) ☐ Poor (<11)

Figure 14.33 Stream study sampling form.

Biotic index

When you perform a river or stream study, you count the number of macroinvertebrates collected in each sample and record the data on a stream study form (see Figure 14.33). The number of organisms in each group is multiplied by a factor that is determined by how sensitive the organism is to pollution. The presence of sensitive organisms is multiplied by a higher number. The more sensitive organisms you have in the sample, the higher the quality of the water in the river or stream. The total number is called the biotic index.

Periodic sampling gives an idea of the overall health of the river or stream. After a storm, there will be a lot of run-off from the areas surrounding the river. How does the run-off affect the biotic index? Is any sewage diverted into a river after a big storm? Is the biotic index different in winter and spring? Sampling provides us with biological data that can be used to answer these questions.

Richness and evenness are components of biodiversity

Biological diversity can be described in two ways: evenness and richness. The number of different organisms in a particular area is the richness. Evenness is how the quantity of each organism compares with the other. Richness only takes into account the kinds of species present in the ecosystem, while evenness take abundance into account.

For example, Table 14.8 compares the numbers of different larvae in samples from two interdunal ponds at the Indiana dunes.

Table 14.8 Dune samples

Larva species	Number of individuals in sample 1	Number of individuals in sample 2
Caddisfly larva	200	20
Dragonfly larva	425	55
Mosquito larva	375	925
Total	1000	1000

Both samples have the same number of individuals, but in sample 2 the numbers are not distributed evenly between the species. Both samples have the same species richness: each has three types of larvae. But they do not have the same evenness.

The individuals in sample 2 are mainly mosquito larvae. A community is not considered diverse if it is dominated by one species.

Analysis of the biodiversity of two local communities

A measure that takes into account both richness and evenness is the Simpson diversity index. To see how this works, let's consider the community of plants on the foredune at the Indiana dunes and the community of plants on the mature dune. Which would you hypothesize is more diverse and why? To calculate the Simpson diversity index, we need the formula

$$D = \frac{N(N-1)}{\text{sum of } n(n-1)}$$

where
D = diversity index
N = total number of organisms in the ecosystem
n = number of individuals of each species

So, for each community we need to know the number of organisms present and the number of individuals of each species present. This information is found by sampling the two dunes with quadrats as follows:

- record the number of plant species in each quadrat
- count the number of individuals of each species
- record the data for each area in tables.

Tables 14.9 and 14.10 record the plant species on the foredune and mature dune of the Indiana dunes.

Table 14.9 Plant species recorded on the foredune of the Indiana dunes

Plant species	Number of individuals, n	$n(n-1)$
Marram grass	50	50(49) = 2450
Milkweed	10	10(9) = 90
Poison ivy	10	10(9) = 90
Sand cress	4	4(3) = 12
Rose	1	1(0) = 0
Sand cherry	3	3(2) = 6
Totals	N = 78	2648

Table 14.10 Plant species recorded on the mature dune of the Indiana dunes

Plant species	Number of individuals, n	$n(n-1)$
Oak tree	3	3(2) = 6
Hickory tree	1	1(0) = 0
Maple tree	1	1(0) = 0
Beech tree	1	1(0) = 0
Fern	5	5(4) = 20

Plant species	Number of individuals, n	$n(n-1)$
Moss	3	3(2) = 6
Columbine	3	3(2) = 6
Trillium	3	3(2) = 6
Virginia creeper	4	4(3) = 12
Solomon seal	3	3(2) = 6
Totals	$N = 27$	62

Using the formula given above, the calculation for the foredune is:

$$D = \frac{78(77)}{2648}$$

$$D = 2.27$$

The calculation for the mature dune is:

$$D = \frac{27(26)}{62}$$

$$D = 11.3$$

Was your hypothesis correct? According to Simpson's diversity index, the mature dune is more diverse even though the total number of plants is less. The mature dune has a higher diversity index because it has a higher number of different species. Periodic sampling of an area and calculation of its Simpson index provides an assessment of the health of an ecosystem.

Investigation into the effect of an environmental disturbance in an ecosystem

Ecological disturbances remove biomass from an ecosystem. The general effect of an ecological disturbance is to shift the community to an earlier stage in succession, which may be more diverse. The aim of this lab it to compare the species diversity in a disturbed area with the species diversity in an area that has not experienced a disturbance, by taking a series of quadrat samples along a transect line in each area.

For this lab, you will take a trip to an ecosystem that has experienced some disturbance. You will use a combination of transect and quadrat sampling techniques to determine plant species diversity along a disturbance gradient. You will also use the same techniques to determine the plant species diversity along a similar gradient that has not been disturbed. Finally, you will calculate Simpson's index for each quadrat in the plant communities along the transect lines and compare the diversity along each transect.

At the site:

- determine the disturbance you will analyse (for example a path made in the dunes, trampling of an area by visitors, a fire, a blow-out, or a windy side of a dune)
- decide when it occurred (how long ago) and whether it is a repeated, intermediate, occurrence, or a one-time disturbance.

Based on the information you have learned about the effect of disturbances on an ecosystem, you can then make a hypothesis about the effect of the disturbance on this part of the ecosystem. Has the diversity of species increased, decreased, or remained the same as a result of the disturbance? Explain why you have made this hypothesis.

The materials you will need are:

- metre-square quadrats
- rolls of thick twine for the transects, or landscape paint
- soil hooks to hold the transect (twine) in place
- a data table to record the plants species present
- graph paper
- a digital camera
- metre sticks
- field guides.

Follow these procedures.

- Work in teams
- Lay out one transect along the gradient of the ecosystem to be examined where a disturbance has occurred (for example along a trampled area).
- Lay out another transect in parallel where no disturbance has occurred.
- Choose the sampling points along the transect.
- Record the distance along the transect at which you located each quadrat (make a map on graph paper).
- Each team should sample at least two transects, with a minimum of 10 quadrats per transect.
- At each quadrat:
  - identify each species within the quadrat
  - if you cannot identify every species, take a picture of it and give it a name of your own, count it, and, if you see it again, it can be counted again (later it may be identified, but the correct name is not necessary to calculate the diversity index)
  - count the number of each species present and record it
  - photograph each quadrat (make sure to label the photograph with the quadrat number and transect name, disturbed or not disturbed)
  - record qualitative observations at each quadrat site, such as the amount of shade and soil type
  - record as much qualitative data as you can about the disturbance.
- Each team can choose its own disturbance, or several teams can do the same disturbance. Working in teams on the same disturbance will allow more data points to be pooled and give a better estimate of the actual diversity.
- Calculate Simpson's diversity index for each quadrat by using the formula that follows and following the example shown based on the data in Table 14.11.

Table 14.11

Species	Number	N(n – 1)
Lyme grass, *Leymus arenarius*	2	2
Sand couch grass, *Elytrigia*	8	56
Marram grass, *Ammophilia arenaria*	1	0
Sea sand wort, *Honckenya*	1	0
European searocket, *Cakile maritima*	3	6
Total (*N*)	15	64

The real cost of damaging nature, it turns out, is at least 10 times greater than the cost of maintaining an ecosystem. Using Simpson's index of biodiversity helps ecologists keep track of the changes in diversity that can indicate problems in an ecosystem.

How do we justify our knowledge? Is an indicator organism sufficient evidence?

NATURE OF SCIENCE

The World Association of Zoos and Aquariums (WAZA) is the lead organization for the world zoo and aquarium community. Its mission is to provide leadership and support for zoos and aquariums. It is dedicated to the preservation of species and involves international cooperation in order to promote conservation of biodiversity around the world.

$$D = \frac{\text{sum of } n(n-1)}{N(N-1)}$$

$$D = \frac{64}{15(14)}$$

$$D = \frac{64}{210}$$

Where D = diversity index, N = total number of organisms in an ecosystem, and n = number of individuals in each species.

$D = 0.3$, which is Simpson index

$1 - D = 0.7$, which is Simpson's index of diversity.

1. Construct a graph showing how Simpson's index of diversity is related to a factor in the ecosystem, such as the distance from the shore to the first dune for both the disturbed and undisturbed areas in the ecosystem.
2. Draw a conclusion a based on the data you (and your classmates) have collected. Restate the data supporting your conclusion. Discuss any uncertainties.
3. Describe how the design of the experiment or data collection method could be improved. Many websites discuss the pros and cons of various sampling methods. What improvements would you make?

As an alternative lab, you could perform a similar investigation of an aquatic environment, such as a river or stream where some disturbance has occurred. Sample benthic organisms along a disturbed and an undisturbed area. Calculate the species diversity using Simpson's index.

Another alternative would be to perform the entire investigation as a class so that repeat samples can be made at each point in the transect or each area of the river or stream. Or either investigation could be performed over time, returning to the site periodically to collect data.

Management of conservation areas

In order to keep the beauty and diversity of a nature reserve, it is important to manage it effectively. Nature reserves cannot just be left to nature. Active intervention is required to restore areas and protect native species. Examples of good management practices are discussed below.

Restoration

Restoration attempts to return the land to its natural state. To restore land on which vegetation has been destroyed may require managers to use active management techniques such as scrub clearance, cutting or burning, and replanting. A UK project is restoring the heathlands within an area designated as a nature reserve in 2007: the Dorset Heathland Project set up in 1989 and completed in 2006, and regular monitoring is ongoing.

The gopher tortoise is a keystone species. Many other species depend on it for survival.

Recovery of threatened species

Threatened species are usually helped when we restore their habitat. Active management maintains the areas needed for the habitat of the endangered species. In a Florida nature reserve, the habitat of the endangered gopher tortoise is being restored. This tortoise lives in deep burrows in a sandhill ecosystem. As many as 350 other animal species live in the burrow with the gopher tortoise. Restoration of

the sandhill ecosystem is necessary for the existence of all these species, not just the gopher tortoise. Some insects are obligates with the gopher tortoise, which means they are rarely found anywhere except in the burrow that the tortoise digs.

Removal of introduced species

Most of the exotic species (species that are not native to an area when it is introduced) that are introduced into an area die out because they do not have adaptations for the local ecosystem. However, when an exotic species can survive and takes over, it can have devastating results. In parts of the UK, plants called rhododendrons have taken over large areas and almost eliminated the native plants in those areas. Active management is needed to remove rhododendrons from nature reserves in the UK. In the southern USA, the kudzu plant is a very aggressive invader. Active management of kudzu requires removing it as soon as it is spotted.

Legal protection against development or pollution

Nature reserves protected by the government or private organizations can prohibit activities that might harm the native animals and plants. Such activities might be extraction of minerals, development of recreational facilities, hunting of animals, or over-use by the public. Active management measures include posting warning signs and using security personnel to ensure the nature reserve is protected from harmful human activities.

Funding and prioritizing

Because all activities require funding, which should take priority? Should funds be used to remove all exotic species, or can we assume most exotics will die out? Should we repair the habitat of a few endangered species, or use the limited funds to maintain the habitat for the majority of organisms? Should we build footpaths for the public even though that will bring destruction to some of the habitat? Increasing public awareness of reserves can help provide the funds needed to support the reserves. Management of nature reserves requires a balance between the health of the ecosystem, maintenance of diversity, and the costs involved.

In situ conservation methods

Nature reserves help endangered species by maintaining their habitat and preventing competition from invasive species. Keeping these organisms *in situ* means putting them in the ecosystem where they belong. Organisms have adapted over hundreds of years to a certain set of conditions. These conditions include the other species present in the ecosystem as well as abiotic factors. It is the goal of *in situ* conservation to allow the target species to continue to adapt to conditions in the reserve without interference from outside influences, such as invasive species and human incursions.

Reserves can be terrestrial (land-based) and aquatic (water-based). Terrestrial reserves can be found in most communities. Lake and pond areas are also common. Marine reserves are rare and are lagging behind in their development. Terrestrial reserves have been around for centuries, but there is no tradition of conservation of species using marine reserves. The ocean is a large ecosystem that needs protection. The same *in situ* strategies as used in terrestrial reserves can be put into practice in a marine reserve.

In situ conservation aims to achieve the following:

- protect the target species by maintaining the habitat
- defend the target species from predators
- remove invasive species
- have a large enough area in the reserve to maintain a large population
- have a large enough population of the target species to maintain genetic diversity.

On occasion, the *in situ* area is unable to protect the targeted species. For example:

- the species is so endangered that it needs more protection
- the population is not large enough to maintain genetic diversity
- destructive forces cannot be controlled, such as invasive species, human incursion, and natural disasters.

Ex situ conservation methods

Ex situ methods are usually used as a last resort. If a species cannot be kept in its natural habitat safely, or the population is so small that the species is in danger of extinction, then *ex situ* methods of conservation are used. There are three methods: captive breeding of animals, cultivation of plants in botanic gardens, and storage of seeds in seed banks.

Captive breeding

Some zoos have large facilities devoted to breeding. They have staff trained in animal husbandry. Breeding programmes capture the interest of the public and can generate new funds for the zoo. The San Diego Zoo in California, USA, has devoted a large part of its resources to captive breeding programmes. The goal of captive breeding is to try to increase the reproductive output of a species and ensure survival of the offspring. Here are some of the techniques used.

- Artificial insemination. If the animals are reluctant to mate, semen is taken from the male and placed into the body of the female.
- Embryo transfer to a surrogate mother. To increase the number of offspring, 'test-tube' babies are produced and implanted in surrogate mothers. Sperm and eggs are harvested from each parent, respectively, and then joined together in a Petri dish. The resulting zygote is implanted in the female uterus. The mothers can be a closely related species.
- Cryogenics. Eggs, sperm cells, and embryos can be frozen for future use.
- Human-raised young. If a mother is not interested or able to care for her young, then staff can hand-raise the young in the nursery of the zoo.
- Keeping a pedigree. If artificial insemination is a common occurrence in the management of a species, it is important that the relatedness of the individuals is known, to keep inbreeding to a minimum.

One problem with captive-breeding programmes is that the introduction into the wild of captive-bred individuals can spread disease to a non-infected wild population. When some captive-bred desert tortoises were introduced to their native habitat, they infected the wild population with a respiratory disease. Another problem is that

animals bred in captivity have not experienced the process of *in situ* learning that their wild relatives undergo. This may put them at a severe disadvantage in the wild.

Mexican gray wolf.

Captive breeding and the reintroduction of an endangered animal species

Captive breeding has helped save the Mexican gray wolf from extinction. At the beginning of the breeding programme only five wolves, of which only one was a female, could be found in the wild. This lone female gave birth to one male and three females. One male and three females were captured and protected at a site for captive breeding. Today's wild Mexican gray wolves can all be traced back to those first wolves used for the captive breeding programme that began in 1981.

In the western USA, during the early 1900s, the most important prey for wolves were bison and moose. However, these prey species were severely depleted by the actions of human settlers. As a consequence, the wolves began preying on sheep and other livestock. Pressure was put on the government to kill the wolves, and bounty programmes were established. Up until 1965, $50 was offered per wolf. As a result, the wolf population was devastated. After the US Congress passed the Endangered Species Preservation Act in 1966, the gray wolf made it on to the endangered species list. Studies in 2004 showed that when wolves were eliminated the elk population exploded, leading to overgrazing of plants, especially along rivers. A significant decline in plant species such as willow and aspen then led to a reduction in beaver and songbird populations. Evidence was collected showing that removal of wolves led to instability in some environmentally sensitive areas.

In 1997, because of the availability of wolves bred in captivity, the USA reintroduced the gray wolf into areas of Arizona and New Mexico. In 2010 there were 59 wolves living in these areas. Radio-tracking methods are used to monitor the population size and health of these important animals. Hopefully, their populations will continue to increase. This is a case where scientists have collaborated with government agencies and wildlife organizations to preserve a species and also to preserve biodiversity in an important ecosystem.

To learn more about the Mexican gray wolf recovery programme, go to the hotlinks site, search for the title or ISBN, and click on Chapter 14: Section C.4.

Botanical gardens

Plants are easily kept in captivity. They have simple needs and usually breeding them is not difficult. About 80 000 plant species are grown in private gardens, arboretums, and botanical gardens all over the world. It is much easier to take care of and breed plants outside their natural setting than it is to take care of and breed animals. One problem with the collections of botanical gardens, however, is that the wild relatives of commercial crops are underrepresented. These plants may have genes that confer resistance to diseases and pests. Adding these wild plant relatives to collections at botanical gardens would provide gene banks for commercial crops.

Zoos play an important role in species preservation internationally. A zoo in South Carolina is supporting the preservation of species from all over the world.

Seed banks

Seeds in a seed bank are kept in cold, dark conditions. Under these conditions, the metabolism of the seed slows down and prevents it from germinating. Seed can be kept this way for decades. Some seeds are grown, allowed to mature, and their new seed

In 2006 the Norwegian government established a global seed bank. The Millennium Seed Bank Project at the Royal Botanical Gardens in the UK aims to safeguard 24 000 plant species from around the globe.

collected. Currently, seeds from 10 000 to 20 000 plant species from all over the world are stored in seed banks.

Biogeographical factors affect species diversity

Species diversity is defined as the number of species and their relative abundance. Three factors influence species diversity: latitude gradient, elevation gradient, and the area effect.

- Latitude gradient is the effect of climate on species diversity. The farther you travel away from the equator, the fewer species you will find. For example, the growing season at the equator is five times longer than in a tundra community. Many more plants have an opportunity to grow in a much longer growing season. The short season of the tundra allows only a few plants to grow.

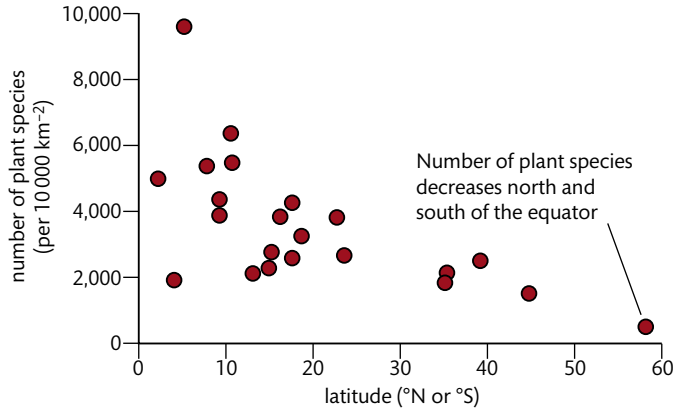


Figure 14.34A Graph of the number of plant species versus latitude. Molles, Jr. 2010, p. 502, Fig. 22.15

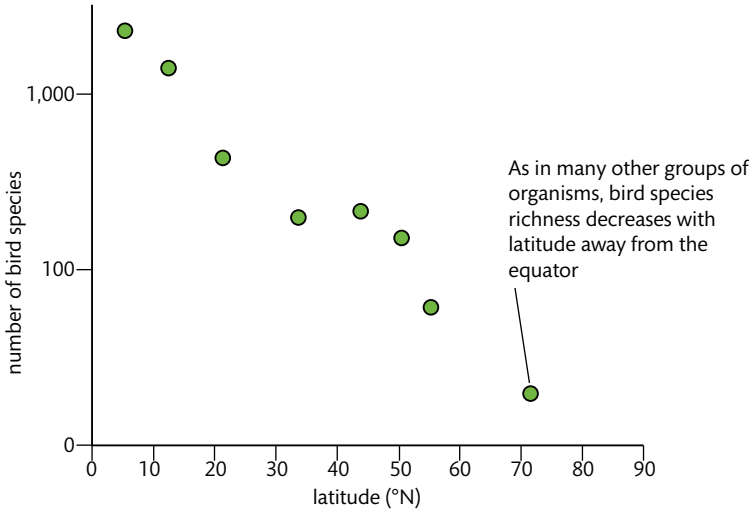


Figure 14.34B Graph of the number of bird species versus latitude. Molles, Jr. 2010, p. 502, Fig. 22.16

- Elevation gradient is the effect of altitude on species richness. As you travel up to higher altitudes, species richness increases until you reach a certain point, and then it declines again. That point is about half way up the elevation gradient and is called the mid-point bulge. At the mid-point bulge the diversity of species is at its greatest. After the mid-point bulge the species diversity declines.
- The area effect is the effect of area on species richness. The larger the geographic area, the more species it can support. Larger areas can offer a greater diversity of habitats

than a smaller area. The area affect concept began with a study of islands: the larger the island, the more diverse the species on the island. The concept of 'islands' has been extended to mean any area that is so isolated it can be considered as an island. For example, a lake can be an island because it is an aquatic environment isolated from other aquatic environments by the surrounding land. A mountain peak, or a woodland fragment isolated from other woodlands by a housing development, can also be considered as islands.

CHALLENGE YOURSELF

In an experiment in 1962, Frank Preston examined the relationship between the areas of islands in the West Indies and number of species.

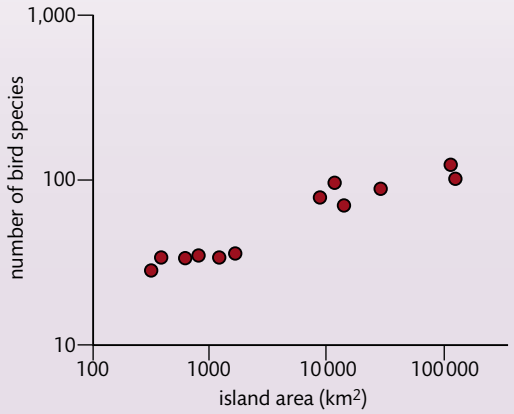


Figure 14.35 Island area versus number of bird species. Molles, Jr. 2010, p. 493, Fig. 22.2 (a)

27 Interpret the results of the Preston experiment from the data shown in Figure 14.35.

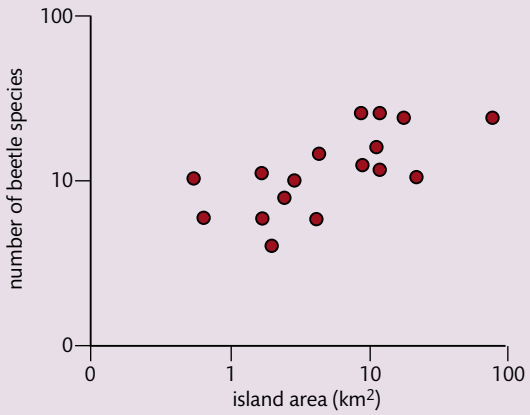


Figure 14.36 The relationship between island area and number of species. Molles, Jr. 2010, p. 493, Fig. 22.2 (b)

28 Look at Figure 14.36, which shows the patterns of species richness in 17 lake islands in Sweden. Interpret the results of this experiment from the data shown on the graph.

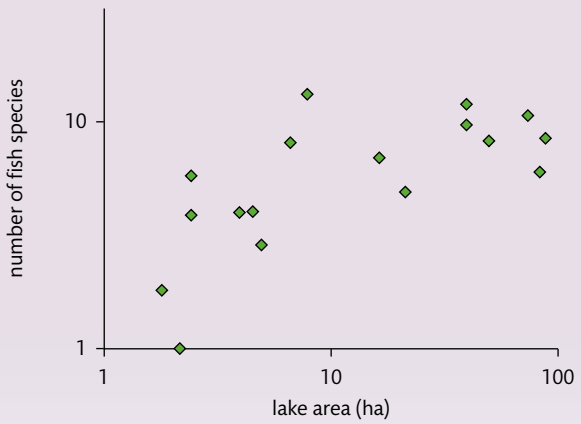


Figure 14.37 Lake area and the number of fish species in the lakes of northern Wisconsin. http://sky.scnu.edu.cn/life/class/ecology/chapter/Chapter22.htm

29 Look at Figure 14.37. Lakes can be considered as habitat 'islands'. Three scientists studied 18 lakes in Wisconsin. Interpret the results of their experiment from the data shown on the graph.

The impact of edge effect on diversity

Edge effect describes what occurs at habitat boundaries where two bordering communities influence each other. Factors that affect the edge can be:

- abiotic, such as more or less sunlight or moisture at the edge of a forest
- biotic, such as the presence of certain predators at the edge.

Does the edge effect promote species diversity in an ecosystem? Some species thrive only at the edges of a habitat because they depend on unique resources that are not present in the interior environment of the habitat. Other species thrive only in the interior environment. High habitat diversity, which includes both the interior and the edge of a habitat, promotes species richness in an ecosystem.

Edge effect plays an important role in the habitat suitability of the western meadowlark and other grassland birds. A study of how the edge effect affects the presence of the western meadowlark provided evidence that species diversity could be increased in the ecosystem if woody plant encroachment was curtailed so that populations of grassland bird species that live at the edge of the woodland could be maintained. A consistent decline in grassland bird populations because of woody plant encroachment decreases species diversity.

CHALLENGE YOURSELF

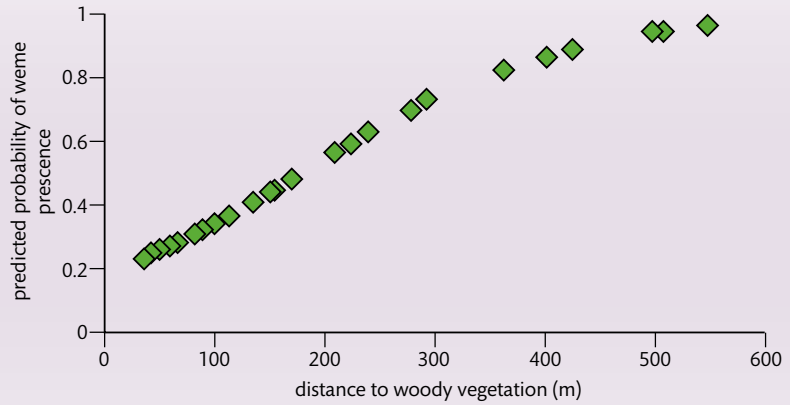


Figure 14.38 Scatterplot of the predicted probabilities from a logistic regression model of western meadowlark presence in relation to the distance to woody vegetation. http://nature.berkeley.edu/classes/es196/projects/2013final/LeeL_2013.pdf

30 What is the effect of woody vegetation on the presence of the western meadowlark?

31 Give a hypothesis regarding the cause for this effect.

32 What other 'edge' factor that might affect grassland birds could be studied?

The western meadowlark.

Exercises

10 Describe a case study of the captive breeding and reintroduction of an endangered animal species.

11 Explain how the edge effect affects species diversity.

12 Describe three factors that influence species diversity.

To find out more about the Mexican gray wolf, go to the hotlinks site, search for the title or ISBN, and click on Chapter 14: Section C.4.

NATURE OF SCIENCE

Avoiding bias: a random number generator helps to ensure population sampling is free from bias.

C.5 Population ecology

Understandings:

- Sampling techniques are used to estimate population size.
- The exponential growth pattern occurs in an ideal, unlimited environment.
- Population growth slows as a population reaches the carrying capacity of the environment.
- The phases shown in the sigmoid curve can be explained by relative rates of natality, mortality, immigration, and emigration.
- Limiting factors can be top down or bottom up.

Applications and skills:

- Application: Evaluating the methods used to estimate the size of commercial stock of marine resources.
- Application: Use of the capture–mark–release–recapture method to estimate the population size of an animal species.
- Application: Discussion of the effect of natality, mortality, immigration, and emigration on population size.
- Application: Analysis of the effect of population size, age, and reproduction status on sustainable fishing practices.
- Application: Bottom-up control of algal bloom by shortage of nutrients and top-down control by herbivory.
- Skills: Modelling the growth curve using a simple organism such as yeast or species of *Lemna*.

Population dynamics

Remember the example of the major volcanic catastrophe at Mount Saint Helens from Chapter 4. From this example, it can be deduced that there are four main factors that affect population size:

- natality, i.e. the number of new individuals after successful reproduction
- mortality, i.e. the number of deaths
- immigration, i.e. the number of individuals arriving from other places
- emigration, i.e. the number of individuals leaving the population.

These trees were knocked down by the Mount Saint Helens blast in 1980.

In the example of Mount Saint Helens, the massive mortality rate as a result of the eruption reduced the populations of birds, trees, mammals, and just about everything else in the vicinity to zero. Emigration before and immediately following the eruption greatly decreased populations in the wider vicinity surrounding the volcano. But today immigration and natality are improving the numbers dramatically.

Shortly after total destruction, life started to repopulate the volcanic region.

Population growth curve

The case of Mount Saint Helens shows that, even from a non-existent or very small population of individuals, there can soon be a dramatic increase in numbers. Over the years, the number of trees and birds near Mount Saint Helens will rise at ever-increasing rates as the organisms reproduce and occupy the available space.

Eventually, when a complete forest has grown again and all the habitats are occupied, the numbers of organisms will stabilize and populations will not get any bigger (see Figure 14.39).

The sigmoid (S-shaped) curve of the graph in Figure 14.39 shows the three stages of population growth.

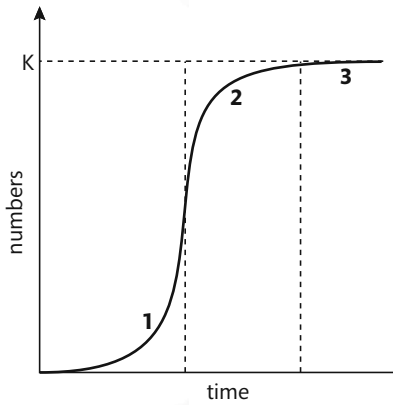


Figure 14.39 Population growth curve. The letter K at the top left is the carrying capacity, which is explained later.

1. The exponential growth phase, also called the logarithmic phase, during which the number of individuals increases at a faster and faster rate.
2. The transitional phase, during which the growth rate slows down considerably, the population is still increasing but at a slower and slower rate
3. The plateau phase or stationary phase, during which the number of individuals has stabilized, and there is no more growth.

So what causes the different phases of the population growth curve?

The exponential phase

In ideal conditions, a population can double in size on a regular basis. Not counting mortality, for example, a population of bacteria can theoretically double its population every few hours: 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, and so on. Without predators, introduced species, such as the cane toads in Australia, can take over habitats with uncontrolled population growth. The reasons for this first phase of exponential growth are:

- plentiful resources, such as food, space, and light
- little or no competition from other inhabitants
- favourable abiotic factors, such as temperature and dissolved oxygen levels
- little or no predation or disease.

The transitional phase

Eventually, after the exponential increase in the number of individuals of a population, some of the factors listed above no longer hold true. This leads to the transitional phase. The causes of the transitional phase are:

- with so many individuals in the population, there is increasing competition for resources
- predators, attracted by a growing food supply, start to move in to the area
- because of the large numbers of individuals living together in a limited space, opportunities for diseases to spread within the population increase.

The plateau phase

Consider the land around Mount Saint Helens slowly being taken over by vegetation. Once all the fertile ground is covered with plants, the space available will be occupied

When asked to draw an S-shaped or sigmoid curve, remember to label it.

Two centuries ago, there were only about 1 billion humans on Earth. Today there are over 7 billion. In which phase of the S-curve is the current human population?

to its maximum. Thus, there is gradually less and less available space for any seeds produced by the plants to germinate, and the number of plants stabilizes.

With increasing numbers of herbivores, there is a limited supply of food. In response to limited food supplies, animals tend to have smaller numbers of offspring.

Predators and disease increase mortality, and the growth curve tends to level off. In this phase, the number of births plus the number of immigrations is balanced by the number of deaths plus the number of emigrations.

Carrying capacity (K)

No habitat can accommodate an unlimited number of organisms: populations cannot continue to grow forever. As you have just seen, there comes a time in the growth of a population when its numbers stabilize. This number, the maximum number of individuals that a particular habitat can support, is called the carrying capacity and it is represented by the letter K (see Figure 14.39).

Consider, for example, a given area of soil in a forest. There is a maximum number of trees that can grow there. This number is attained when enough trees are present to catch all the sunlight, leaving every square metre of the forest floor in shade. New tree seedlings trying to grow under the adult trees will have difficulty getting sunlight.

The penguins in Antarctica may be facing many limiting factors in their habitat.

But many young trees can store up energy for years with very little vertical growth, until a big tree dies, leaving a hole in the canopy. The young trees then race up towards the opening to take the old tree's place. Those that lose this race usually die; and for a young tree to join the mature population, an old tree must die and free up some space.

Limiting factors that define the carrying capacity of a habitat include:

- availability of resources, such as water, food, sunlight, shelter, space, and oxygen (the latter notably in aquatic habitats)
- build-up of waste, such as excrement and excess carbon dioxide
- predation
- disease.

Many biologists, environmental groups, economists, and governments wonder what the carrying capacity of Earth is for the human population. Will the number of people continue in an exponential growth phase, or will diseases, climate change, or competition for resources lead to a transitional phase or a plateau? Only time will tell.

Limiting factors can be top down or bottom up

The limiting factors that define the carrying capacity of a population can exert top-down control, such as predators,

or can be a source of bottom-up control, such as nutrients. This concept is illustrated by the example in the Challenge yourself.

CHALLENGE YOURSELF

The study of a tropical coral reef revealed the effects of top-down and bottom-up limiting factors. The bottom-up limiting factor was the nutrients that increased the algal blooms that negatively affected the coral. The top-down limiting factor was the fish that ate the algae, so keeping the coral reef healthy. Two study sites on the coral reef, A and B, were isolated for 24 months and conditions were manipulated. Controlled experiments were performed pairing high and low herbivory (the amount of algae eaten by fish) with high and low nutrient levels. See Table 4.12.

Table 14.12 Mean percent cover (standard error) of benthic functional groups colonizing clay diffusers following 24 months under reduced and elevated nutrients in low- and high-herbivory study sites ($n = 4$)

Functional groups	Study site A (low herbivory) Nutrients		Study site B (high herbivory) Nutrients		Significant differences ($p < 0.05$)
	Reduced A	Elevated B	Reduced C	Elevated D	
Crustose corallines	41.2 ± 4.6	1.8 ± 1.8	<0.1	71.7 ± 3.0	D > A > B, C
Frondose macroalgae	20.8 ± 4.3	63.7 ± 8.2	0.6 ± 0.3	16.9 ± 4.1	B > A, D > C
Algal turfs	37.1 ± 3.9	14.5 ± 4.7	<0.1	22.1 ± 2.9	A > D > B > C
Predicted dominants	Turfs	Macroalgae	Corals	Corallines	

Three types of algae were included in the study, as shown in Table 14.12:

- crustose corallines, which are beneficial algae that help the coral build the reef
- frondose macroalgae, which are fleshy and filamentous, and can overgrow the coral and prevent healthy reef building because of their algal blooms
- algal turfs, which are microalgae and their blooms are also detrimental to reef building.

Parrot fish.

The herbivorous fish were parrotfish and surgeonfish.

The question posed by the study was how the effect of top-down herbivore and bottom-up nutrients affected the competition of harmful and beneficial algae. The percentage of reef cover by each type of algae was a measure of their success.

33 For study site A, compare the mean percentage cover of all three alga types with reduced and elevated nutrients. What were the resulting effects on the coral?

34 For site A, the prediction was that macroalgae would be dominant in the competition for percentage cover with elevated nutrients. Was that prediction correct? Give evidence to support your answer.

35 Describe a benefit to the coral reef that occurred over the 24 months at site D.

36 Explain the conditions under which eutrophication-induced microalgae blooms decreased the growth of the reef-building corals.

Surgeon fish.

Natality, mortality, immigration, and emigration, and the sigmoid growth curve

The equation below describes the relationship between natality, mortality, immigration, and emigration, and change in population density

Change in population density =	**(natality + immigration) minus (mortality + emigration)**

The sigmoid growth curve is an idealized version of population growth. It tells us that, in the exponential phase, natality is high and mortality is low. This causes the population to grow geometrically. However, when space and food begin to be limiting factors, then natality is lower and mortality higher. At the plateau of this curve, natality and mortality are equal, and emigration and immigration are equal. The ideal population is in dynamic equilibrium at the carrying capacity of the environment (the plateau on the graph).

Factors that change the sigmoid growth curve

Four factors that influence change in the sigmoid growth curve are:

- abiotic
- biotic
- density independent
- density dependent.

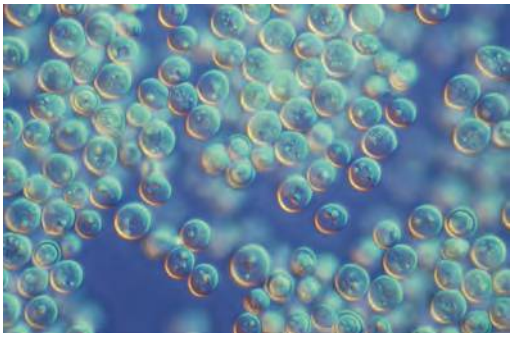
An example of an abiotic factor is temperature. For example, cold temperatures could increase mortality in an exponentially growing population of fruit fly larva. The population may have been growing exponentially until a sudden freeze. This causes natality to immediately decrease and mortality to dramatically increase.

An example of a biotic factor is the cane toad that was introduced into Australia for biological control. Because the cane toads are toxic, no predators can eat them. The population of cane toads has grown exponentially. Natality was high and immigration was high where they were introduced to eat cane beetles. Mortality was low. As they reached the carry capacity of the environment in some areas, emigration began. Some of the cane toads moved to other areas in Australia. In those new areas the immigration started a new population explosion (increased natality).

A density-independent factor affects all of the population equally. For example, a hard frost will kill all leaf hoppers equally, whether the population is 10 leaf hoppers or 100 leaf hoppers. Natality will fall to zero and mortality to 100%. Hurricanes or wildfires are other examples of density-independent factors that affect a population growth curve.

A density-dependent factor affects large populations and small populations differently. For example, a large, dense population will exhibit a greater increase in mortality with the spread of a contagious disease than a small population that is not so densely crowded. A larger, dense population is more likely to experience emigration as individuals move to other areas in search of more food and space. Predators may emigrate to an area of high prey density; this will increase mortality and reduce natality in that area.

You can see how environmental factors affect birth, death, immigration, and emigration. These factors change the shape of the idealized curve.



Yeast cells.

Modelling the growth curve of yeast

The yeast *Saccharomyces cerevisiae* is a single-celled organism that reproduces by budding. In this lab you will watch the population growth of yeast over several days. Would you predict exponential growth for the first few days? It will be interesting to discover whether the yeast will reach the carrying capacity of the environment that you design. This can be done as a class activity.

Day 1 (Monday)

1. Pour 20 ml of nutrient broth into a clean 100-ml beaker.
2. Obtain 20 ml of the yeast culture from your teacher in another 100-ml beaker.
3. Obtain five test tubes and place them in a test tube rack.
4. Using a sterile plastic pipette, place 3 ml of nutrient broth in each test tube.
5. Next add 3 ml of yeast solution to each test tube.
6. Cover each tube tightly with parafilm (clingfilm). Shake the tubes hard for 30 seconds and then lift the parafilm to allow any gas to escape.
7. Number the tubes (1–5) with a glass marking pencil.

Day 2 (Tuesday)

1. Add 5 drops of methylene blue solution to tube number 1.
2. Replace the parafilm and invert the tube, shaking gently.
3. Obtain a microscope, slide, and cover slip. Remove a small drop of the culture from the middle of the tube. Place a drop on your slide and gently add a cover slip.
4. Focus first with low power and then switch to high power.
5. Living cells will be white because they change methylene blue to white. Count the number of live yeast cells you can see in one the field of view of the microscope. If a cell is blue, it is dead so don't count it. If a cell is budding, count it as two. If the field is too full to count, mentally divide it into fourths or eighths, count the cells within that fraction, and multiply that number to get the count as accurately as possible for one field of view. Now you can determine your accuracy, for example ±10 yeast cells. Count a second field of view if time permits.

On days 3 and 4, repeat the above procedure. You may have to let the culture sit two days over the weekend without being counted. Day 5 should be the last count and should be on Monday.

Use your team data and also your class data, if it is available, to determine whether the yeast has shown the sigmoid growth curve that would be expected based on the theory of population growth over time.

Alternative lab 1

Grow duckweed or *Lemna* in pond water over several months under grow lights. Collect population samples periodically to determine whether there is exponential growth that slows and finally reaches the carrying capacity of the container in which the plant is growing.

Alternative lab 2

Begin with a vial of fruit fly media and ten flies in a vial. Count the population of flies in that vial every week for 8 weeks. Your vial can be considered as a sample of the entire population. Using class data will make your results more accurate.

Use of capture–mark–release–recapture method

The capture–mark–release–recapture method is a sampling technique that enables you to estimate the number of animals in an ecosystem. The technique involves catching some of the population and marking them. The marked animals are released back into the ecosystem and allowed to mix with the others in the population. A second sample of the population is then captured. Some in the second sample will be marked and some will be unmarked. The proportion of marked to unmarked individuals in the second sample is the same as the proportion of the originally marked individuals to the whole population. Here is the formula:

$$\frac{\text{number marked in the second sample } (n_3)}{\text{total number caught in second sample } (n_2)} = \frac{\text{number marked in the first sample } (n_1)}{\text{size of the whole population } (N)}$$

or

$$\frac{\text{population size } (N)}{} = \frac{(n_1 \times n_2)}{n_3}$$

Worked example

Suppose you capture and mark 100 grasshoppers and release them back into the ecosystem. Then you capture another sample of 100 grasshoppers and find ten of them are marked. Estimate the population size.

Solution

$$\frac{1}{100} = \frac{100}{N}$$

or

$$N = 100 \times \frac{100}{10}$$

$$N = 1000$$

The capture–mark–release–recapture method does have limitations:

- marking the animals may injure them
- the mark may make an animal more visible to predators (if marked animals are eaten, your second sample will not be reliable)
- it assumes that the population is closed, with no immigration or emigration (very few populations are closed).

Does the method really work? You can try it at home with popcorn kernels.

- Count out 200 popcorn kernels and put them in a bag.
- Remove 40 kernels and mark with a permanent marker.
- Put the marked kernels back into the bag and shake the bag.
- Remove 40 more kernels and record how many are marked.
- Use the formula to determine population size.

Did you come close to 200? One sample is not enough data. To be accurate, you should repeat the sampling technique at least five times (10 times is even better). Now average your the results. How close are you to 200 now?

Estimating the size of commercial fishing stocks

How do scientists really know what is in the ocean or a lake? The following are methods used to predict the size of commercial fish stocks.

Studying catches

For the North Atlantic Ocean, scientists from the International Council for Exploration of the Sea (ICES) sample fish catches at seaports. Data are collected on the type of fish, age, length, and breeding condition.

Gathering information from fishermen

Who is better informed about the number of fish caught than the people who catch them? Scientists from ICES also collect information on-board fishing vessels. Their tasks on-board include:

- recording the number and kinds of fish that are thrown back
- tagging and releasing some fish
- developing questionnaires for fishermen about their perception of the catch
- reviewing the ship logbooks, which provide data on catch per unit effort (increased effort for the same catch indicates that fish are getting scarcer).

Global demand for fish has doubled in less than 30 years because of human population growth in poor countries and a matching increase in demand for fish in those countries.

Using research vessels

Research vessels collect information in a variety of ways.

Casting nets in hundreds of selected locations

Sampling with nets is called trawling. Scientists need to make random samples, not visit locations where fish are known to congregate. They must be careful to use the same sampling methods every time they sample so that results can be compared.

Using sound to monitor fish populations

An echo sounder reads information from a pulse that it sends into the water. The returning echo indicates the presence of a shoal of fish. After doing hundreds of soundings, the scientists reading the data can even tell what species of fish has been located. To verify the type of species, a trawl is done and a sample collected. The remote sensing hydroacoustic method can determine both the number and biomass of fish populations.

Calculating the age of fish in a population

Knowing how many younger fish and how many older fish are present is very useful. Too few young fish indicates lack of spawning, and too few old fish may mean that over-fishing is taking place. One method of calculating age is to measure the rings in the otoliths (ear bones) of a fish. As a fish grows, new material is deposited in its ear bones. When the rings are counted under a microscope, it indicates the age of the fish. Another method is to measure the rings of fish scales.

Using coded wire tag detectors

Fish populations can be marked by attaching tags to the fish. As the fish are recaptured, the total population can be estimated. This is similar to the mark–release–recapture method. The Michigan Department of Natural Resources (MDNR) puts a coded microscopic wire tag in the nose of chinook salmon and lake trout that have been planted (stocked) in the Great Lakes. Tagged fish also have their adipose fin clipped. Recollecting fish with tags helps the MDNR evaluate the behaviour and survival of these fish. In order to read the tag, a hand-held detector is used. The fish must be caught and the tag read by hand. In small cities around the Great Lakes there is a programme for fishermen to help with the sampling. If they catch a fish that is missing the adipose fin, they place the head in a special box provided by the MDNR. Fish heads are collected and each is then checked with the handheld detector for the presence of a tag.

Analysing data using mathematical models

Mathematical models are used to turn all of the data into a form that can be used by the fishing industry and governments to plan the future of the fish in our oceans and lakes.

Evaluating these methods

- Gathering data from fishermen: data should be gathered from several sources to cross-check the fishermen's individual data. Data from log books should be cross-checked against sales receipts.
- Computers: using computers on large fishing vessels with automatic data-logging software improves the counting accuracy and communication of total fish counts to a central location.
- Observers: direct observation on fishing vessels, in processing plants, and in fish markets can provide scientists with helpful data for improving the count of fish being caught sold and processed.
- Sound-tracking equipment placement: a new modelling method has been designed to predict where sound-tracking equipment should be located. Previously, the location was random. Now researchers can predict with 95% accuracy where certain fish will be located, so they can be more easily counted.

Improvements in fish stock estimates will ensure that we do not go over the maximum sustainable yield (MSY) for the fish populations. The MSY is the highest proportion of fish that can be removed from the total population without jeopardizing the maximum yield in the future.

If the fish stock is too small, there are not enough adult fish to produce sufficient young fish. Fishing from a stock that is too small leads to over-fishing of the stock. If the fish stock is too large, annual reproductive rates may be low because of competition for food. Between these two extremes is a fish stock size that can produce the MSY. To maintain the MSY, enough fish stock must be left to spawn a new population of healthy fish.

TOK

New paradigms are emerging to guide the management of marine fisheries. The focus is on solving the problem before it begins, rather than solving the problem after it has already decimated the fishing stock.

Black grouper drifting through a coral reef.

The effect of population size, age, and reproductive status on sustainable fishing practices

Commercial fishing is not the only fishing pressure exerted on fish populations. In a study performed by the US Department of the Interior on Key Biscayne National Park in southern Florida, a large rise in the number of people living and fishing in Florida has negatively affected the population of fish.

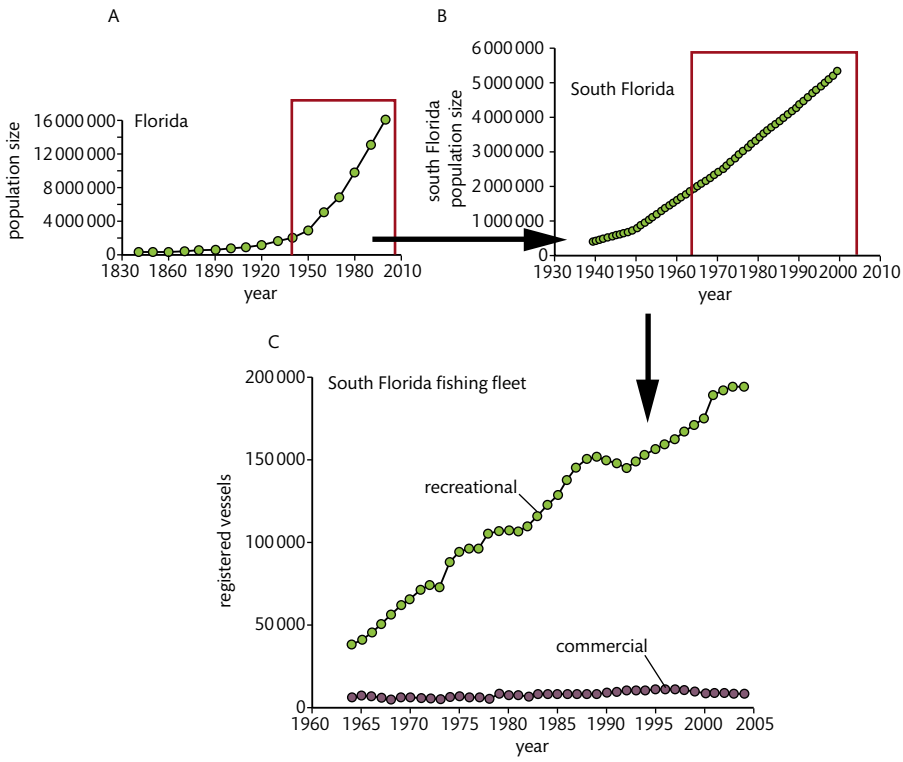


Figure 14.40 Growth of: (A) Florida's human population from 1840–2000; (B) south Florida region human population 1940–2000; and (C) south Florida commercial and recreational fishing fleets from 1964–2004.
Ault et al. 2007, Fig. 1

The aim of the study was to give the fishery management in Key Biscayne strategies for promoting sustainable fishing practices. The study concluded that fish population densities can be improved by regulations aimed at both recreational and commercial fishers. Such as:

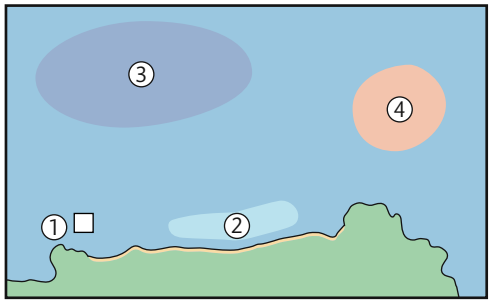
- a reduction in the mortality rate of the fish, by reducing bag limits (the number of fish a fisherman is allowed to be catch each day)
- an improvement in reproductive status, by shifting the harvest to larger fish sizes, thus increasing the size limits for fish that can be kept and the fish have a longer time to reproduce before they can be caught and not released back into the ocean
- an improvement in the age of fish caught, thus increasing spawning potential, which would also be accomplished by increasing size limits, again because the older the age of the fish that can be kept and not released, the more time it has to reproduce. Catching younger and smaller fish lowers reproduction rates.

Establishing marine reserves and no-catch zones can improve biodiversity and increase fish stocks in areas that are protected. The journal *Science* has published small-scale experiments that show that less diverse ecosystems produce less yield. The implication is that it is a loss of biodiversity that is driving the reduction in fish stocks. The *Science* article quotes other studies that show that having protected zones, like marine reserves, restores biodiversity, and also restores populations of fish outside the protected areas (see Figure 14.41). It is difficult to enforce and monitor such regulations. Often, international trust does not exist to keep these practices functioning.

Figure 14.41 Fisheries and biodiversity: the evidence.

3 Open ocean fisheries records show widespread decline of fisheries. In 2003, 29% of fisheries were collapsed. Biodiverse stocks fare better.

1 Experimental guidance shows that lowering the diversity of an ecosystem lowers the abundance of fish.



4 No-catch zones show an average 23% improvement in biodiversity and an increase in fish stocks around the protected area.

2 Coastal fisheries records show extensive loss of biodiversity along coasts, with the collapse of about 40% of species. About a third of coastal fisheries are now useless.

CHALLENGE YOURSELF

Harvesting strongly affects long-lived fish such as the black grouper, *Mycteroperca bonaci*, which lives to a maximum age of 33 years. As exploitation (fishing) increases, there is a significant effect on the number of mature and larger size grouper.

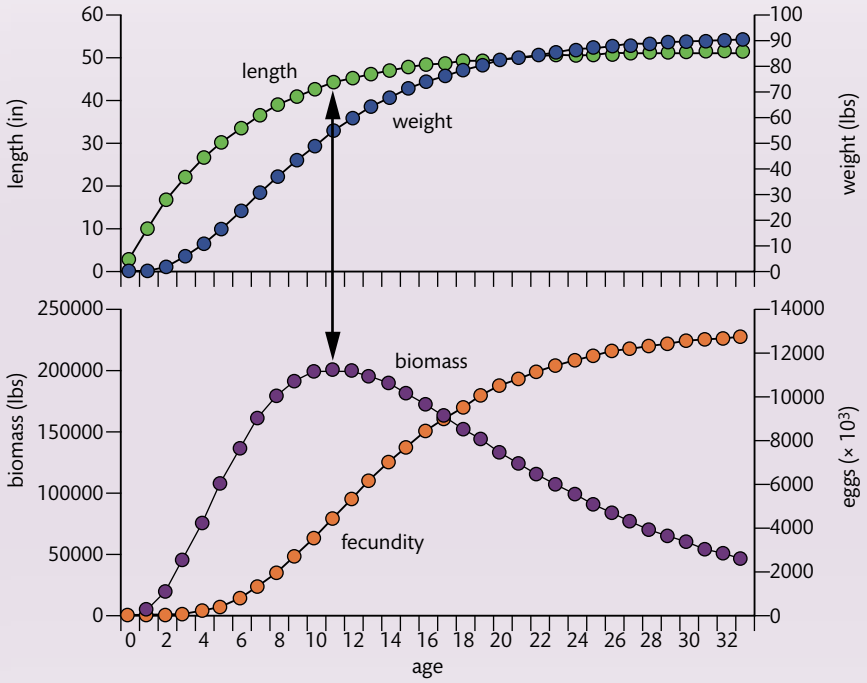


Figure 14.42 Demographic and population-dynamic relationships for black grouper, *Mycteroperca bonaci*. Ault et al. 2007, Fig. 8

37 Use Figure 14.42 to explain what is happening to the population of large, mature groupers.

38 How does this affect the reproductive (fecundity) status of the grouper populations? Explain your answer.

39 The current size limit for black grouper is 24 inches. The current bag limit is two grouper per person per day. In 1995, less than 2% of surveyed fishing trips kept more than one grouper. What does that suggest to you about the size of the fish in the grouper population?

40 Suggest a solution to this problem using evidence from the graphs.

41 How can you convince recreational fisherman that this solution is necessary?

Exercises

13 Describe the factors that can influence the sigmoid growth curve of a population.

14 Compare top-down and bottom-up limiting factors that can affect population growth.

15 Evaluate one method used to determine the size of commercial stock of marine fish.

C.6 Nitrogen and phosphorus cycles

Understandings:

- Nitrogen-fixing bacteria convert atmospheric nitrogen to ammonia.
- *Rhizobium* associates with roots in a mutualistic relationship.
- In the absence of oxygen, denitrifying bacteria reduce nitrate in the soil.
- Phosphorous can be added to the phosphorous cycle by application of fertilizer, or removed by the harvesting of agricultural crops.
- The rate of turnover in the phosphorous cycle is much lower than the nitrogen cycle.
- Availability of phosphorous may become limiting to agriculture in the future.
- Leaching of mineral nutrients from agricultural land into rivers causes eutrophication and leads to increased biochemical oxygen demand.

Applications and skills:

- Application: The impact of waterlogging on the nitrogen cycle.
- Application: Insectivorous plants as an adaptation for low nitrogen availability in waterlogged soils.
- Skills: Drawing and labelling a diagram of the nitrogen cycle.
- Skills: Assess the nutrient content of a soil sample.

NATURE OF SCIENCE

Assessing risks and benefits of scientific research: agricultural practices can disrupt the phosphorus cycle.

The nitrogen cycle

Bacteria play a hugely important part in the processes by which nitrogen is continuously recycled through the environment. Roles of bacteria in the nitrogen cycle are summarized in Figure 14.43 and the accompanying numbered points.

Rhizobium lives in the root nodules of legumes and fixes atmospheric nitrogen. These bacteria are symbiotic and receive carbohydrates and a favourable environment from their host plant.

Nitrobacter lives in well-oxygenated soils and changes nitrites into nitrates, which are useable by plants.

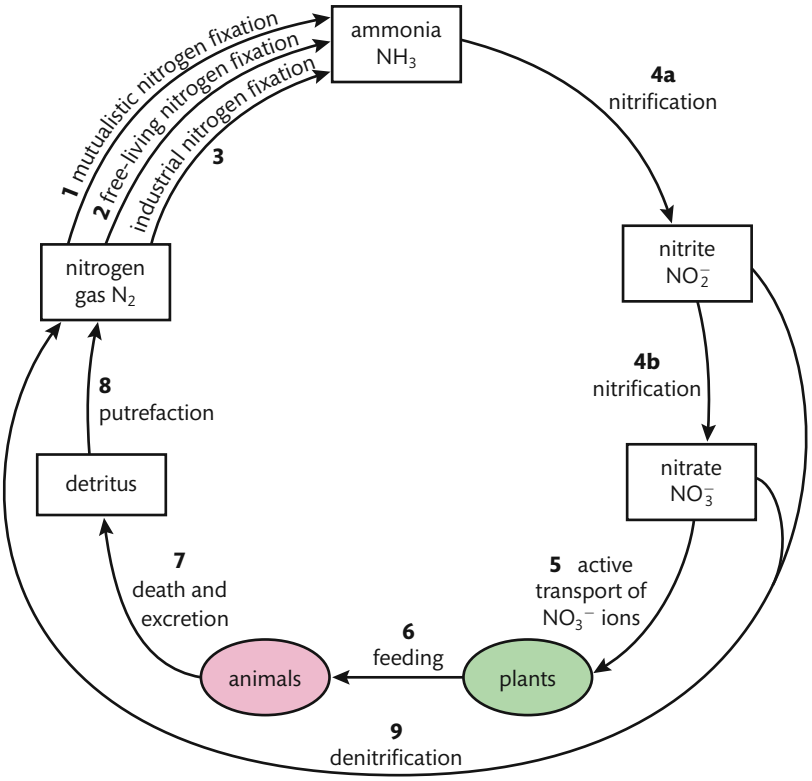


Figure 14.43 Steps of the nitrogen cycle and roles of bacteria.

1 Mutualistic nitrogen fixation. Certain bacteria form a symbiotic relationship with a host plant and fix nitrogen for it, e.g. *Rhizobium* lives in symbiosis with legumes (beans, peas, and clover).

2 Free-living nitrogen fixation. Nitrogen-fixing bacteria, e.g. *Azotobacter*, live freely in the soil and do not need a host.

3 Industrial nitrogen fixation. Burning fossil fuels to produce fertilizer is an important source of fixed nitrogen.

4a Nitrification. Oxygen is needed to turn ammonia into nitrites by bacteria in the soil, e.g. *Nitrosomonas*.

4b Nitrification. Oxygen is also required to change nitrites into nitrates by soil bacteria, e.g. *Nitrobacter*.

5 Active transport of nitrates. Nitrates are actively transported by plants (using ATP for energy) into their roots.

6 Plants and animals. Plants use nitrates to make their own proteins. This process is called assimilation. Animals feed on plants, digest, and rearrange plant proteins to make their own proteins.

7 Death and excretion. The waste products of digestion and dead bodies of plants and animals are full of molecules containing nitrogen.

8 Putrefaction. Decomposers such as bacteria and fungi break down complex proteins, and release nitrogen gas into the atmosphere.

9 Denitrification. Some bacteria, e.g. *Pseudomonas denitrificans*, remove nitrates and nitrites, and put nitrogen gas back into the atmosphere.

Conditions that favour nitrification and denitrification

Nitrification occurs as a result of the actions of two bacteria. *Nitrosomonas* converts ammonia (NH_3) into nitrite (NO_2^-). Then *Nitrobacter* changes nitrite (NO_2^-) into nitrate (NO_3^-), which is useable by plants. These are aerobic reactions carried out by two autotrophic bacteria that are beneficial to the environment. The conditions required for nitrification are:

- available oxygen (the reaction is aerobic)
- neutral pH (preferred by the two bacteria)
- warm temperature (preferred by the two bacteria).

Denitrification is the conversion of nitrates to nitrogen gas. This takes place in anaerobic conditions by autotrophic bacteria. Bacteria such as *Pseudomonas denitrificans* use NO_3^- instead of oxygen as the final electron acceptor. The conditions required for denitrification are:

- no available oxygen (e.g. flooding or compacted soil)
- a high nitrogen input.

Denitrification is not good for soils because it removes the beneficial nitrates needed by plants to make proteins. Denitrification also destroys the ozone layer. Another product, nitrous oxide (NO), can contribute to global warming, as it is a minor greenhouse gas.

Release of raw sewage and nitrate fertilizer into rivers

As societies become urbanized (living in towns), the common problem of waste disposal grows, particularly in relation to sewage. A related problem is the run-off of excess nitrate fertilizer from farms, golf courses, and lawns, which flows into rivers and streams. Effective waste management is a rising cost in our society, but these problems must be solved in order to prevent dire consequences.

Releasing raw sewage into water systems was common until, in the 1850s, it was shown that cholera was transmitted by water contaminated with faeces. *Escherichia coli* (an intestinal bacterium) is frequently in the news in the western world for causing outbreaks of food poisoning: it is spread by contaminated water and lack of hand-washing. The Ganges River in India is the site of a hugely popular festival where people ritualistically bathe in the river, which is now contaminated with human faeces. There are many, many places in the world where a clean water supply is desperately needed. Pathogens should not be found in bathing and drinking water, or water used to irrigate crops.

Nitrates may not sound as dramatic a problem as raw sewage, but they can be disastrous to ecosystems. The presence of excess nitrates and phosphates in rivers and streams is termed eutrophication. The process of eutrophication proceeds as follows:

- high nitrates and phosphates fertilize the algae present in water
- there is increased growth of algae (called an algal bloom)
- the algae are decomposed by aerobic bacteria, which use up the oxygen in the water (a high use of oxygen is called biochemical oxygen demand, BOD)
- the water becomes low in oxygen (deoxygenation), and fish and other organisms that need oxygen die.

Crop rotation is the process whereby a series of different types of plants are grown in the same area. This allows nitrogen to be replenished in the soil by plants such as beans, which have nitrogen-fixing bacteria in their root nodules.

A pitcher plant.

Insectivorous plants in waterlogged soils with low nitrogen

Plants need nitrogen for protein synthesis and to make new DNA molecules. You may remember that part of DNA is made from nitrogen bases like adenine and thymine. Insectivorous plants such as the pitcher plant derive their nitrogen from trapping and consuming insects. Pitcher plants are adapted to living in waterlogged areas where nitrogen is lacking. Pitcher plants also need a large amount of the nitrogen-rich enzyme rubisco. This enzyme is involved in the first major step of making glucose during photosynthesis (see Chapter 2).

Impact of waterlogging on the nitrogen cycle

Healthy plant roots need oxygen. Soil that is waterlogged is so saturated with water that oxygen cannot get into the soil. Waterlogged soils create anaerobic soil conditions with no oxygen. Anaerobic conditions facilitate the growth of denitrifying bacteria, which convert the nitrates needed by plants back to gaseous nitrogen. Waterlogged soils become nitrate depleted and plant (crop) growth is reduced significantly. Waterlogging can interfere with the normal cycle of nitrogen moving from the atmosphere to plants as nitrates and then back to the atmosphere. If waterlogging becomes a large problem because of climate change causing floods, food crops could be seriously affected.

Spreading fertilizer.

The phosphorous cycle

Phosphorous is an essential element in living systems. For example, phosphorous forms part of ATP, RNA, DNA, and phospholipid molecules. Phosphorous is not very abundant in the biosphere and there is not a substantial atmospheric pool of phosphorous as there is for carbon and nitrogen. The largest quantity of phosphorous is found in marine sediments and mineral deposits. Sedimentary rocks that are rich in phosphorous are mined for fertilizer and applied to soils. When crops are harvested, the phosphorous is removed. The only method of replacing it again is to add more fertilizer. Unlike nitrogen, which can be fixed by bacteria and added back to the soil, phosphorous is not recycled easily. Composting is one method of recycling phosphorus; however, composting is not easily done on a large scale.

The rate of turnover in the phosphorous cycle is low

Phosphorous is slowly released into ecosystems from weathering rocks. As it is released it can be absorbed by the roots of plants or washed into rivers. The phosphorous that is washed away eventually finds its way into oceans, where it remains in a dissolved form until finding its way into ocean sediments. The sediments eventually form sedimentary rocks. Sedimentary rocks that slowly wear away make phosphorus available again to plants.

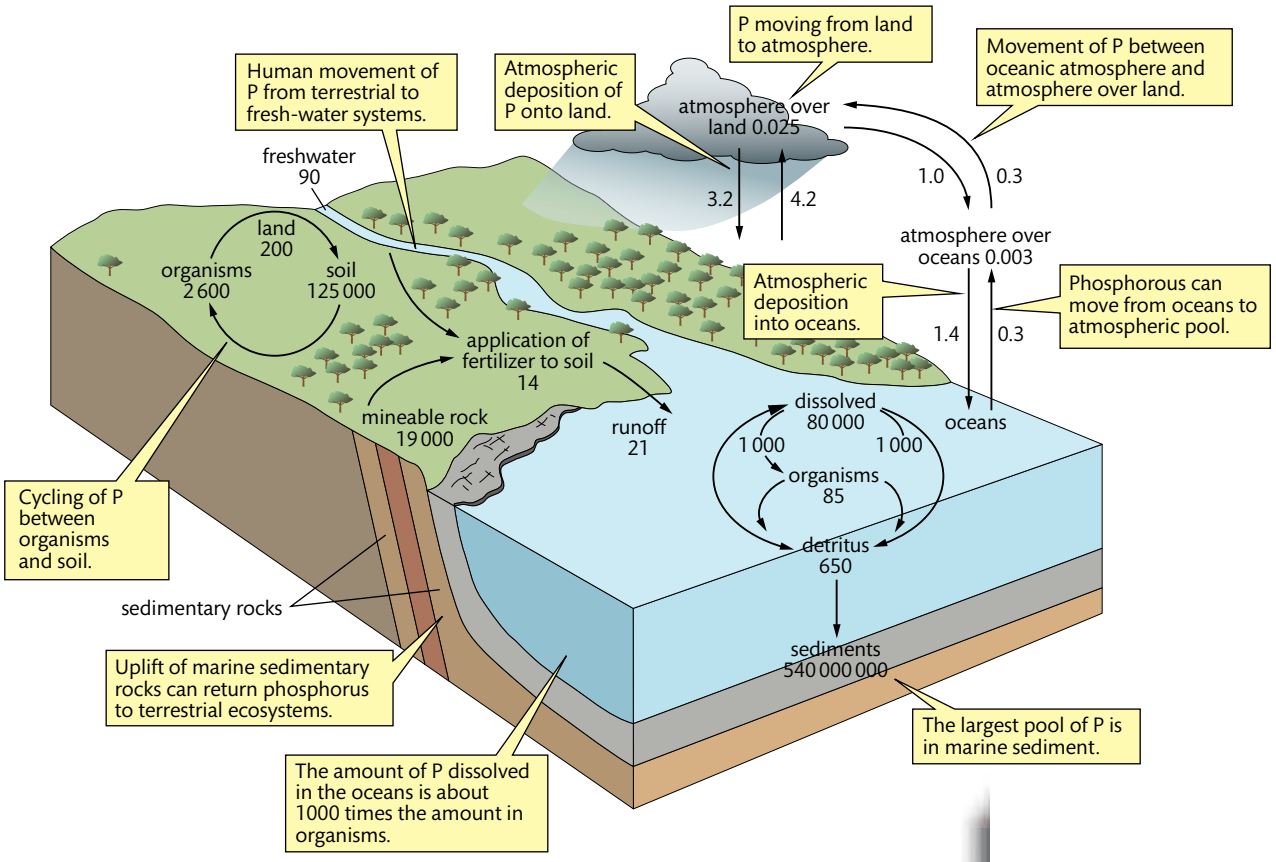


Figure 14.44 The phosphorus cycle.
Molles, Jr. 2010, Fig. 19.2

The availability of phosphorous may become limiting to agriculture

Currently, the major use of phosphate is in chemical fertilizer, which is used in modern agricultural production. Mining phosphorous for fertilizer is consuming the phosphorus more quickly than geological cycles can replace it, and crops remove phosphate from the soil. Most of the world's farms do not have enough phosphate. Phosphate reserves are limited, and it is estimated that the world phosphate reserves will only last another 50–100 years. Feeding the world's increasing population requires more and more crop production, and more and more phosphate. This is accelerating the rate of the depletion of phosphate reserves.

According to an article in *Scientific American* in 2009, the USA is the second largest producer of phosphates after China; 19% of phosphate comes from the USA, and the source of all the USA phosphate mining is from one area in Tampa, Florida. The USA may run out of this accessible, domestic source in a few decades. As the phosphate reserves run out, food prices are expected to increase as rock phosphate reserves become more and more expensive to extract. In the long term, phosphate will have to be recycled from animal and human waste.

Phosphate rock grinding mill at a phosphate mine.

The phosphorous cycle is being disrupted by:

- **massive human use**
- **the difficulty in recycling phosphorous**
- **the difficulty in obtaining phosphorous**
- **the short supply of phosphorous.**

The disruption may affect agriculture in the future and raise food prices. Improvements could be made if we are willing to change agricultural practices and including composting and recycling of human and animals waste.

NATURE OF SCIENCE

Assessing the risks and benefits of scientific research: should we grow fuel crops or food crops with our limited phosphorous reserves? The production of biofuels can only compound the problem. The phosphorous needed to grow food is being used to grow biofuel.

Testing soil samples for nutrients

1. Purchase a soil testing kit that can determine the pH, nitrogen, phosphorous, and potassium levels of your soil. The kit should determine your pH in 0.5 increments from pH 4.5 to 7.5. The kit should also determine nitrate, phosphate, and potassium levels in 5-unit increments.
2. Dilute your soil sample 1:5 with distilled water.
3. Perform each test according to the directions in the soil test kit.
4. Record the data.

Possible variations are listed below.

Test three different areas with different types of soil, e.g. farmland, parkland, and a garden. Repeat each test five times. Calculate an average and standard deviation for each site for each nutrient and pH. Graph the data. Use an online calculator to calculate a Student's *t*-test to examine whether the differences measured are significant.

Test farmland or a vegetable garden over a whole season to determine whether the nutrient content and pH change from one season to the next based on the crops present or harvested, or whether the farmland/garden has just been fertilized. Repeat the procedure so that each test is done five times.

Use class data to build up a larger database. Plan the procedure as a class so that all the variables are controlled and the data collection process is exactly the same for each student.

To use a *t*-test calculator, go to the hotlinks site, search for the title or ISBN, and click on Chapter 14: Section C.6.

Exercises

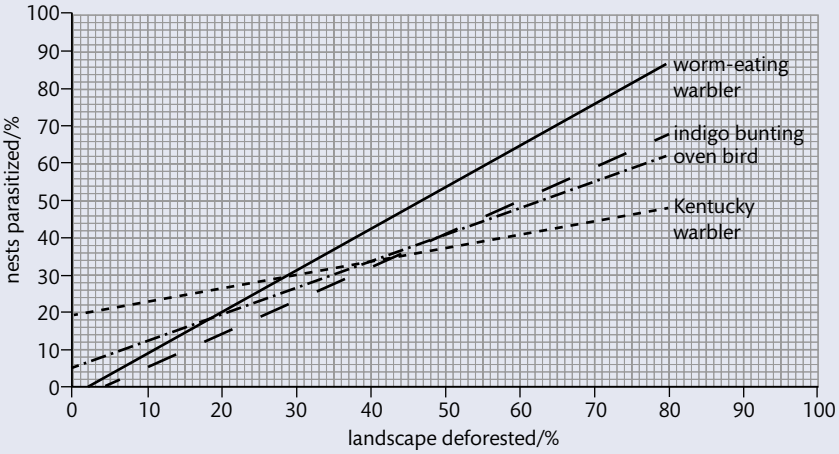
16 Explain the effect of denitrifying bacteria.

17 Compare and contrast nitrogen and phosphate as minerals necessary for plant growth.

18 Describe the three methods of fixing nitrogen in the nitrogen cycle.

Practice questions

1 The brown-headed cowbird, *Molothrus ater*, is a parasitic bird that lays its eggs in the nests of other species. The parasitized hosts often raise the resulting cowbird offspring as their own. The true offspring may starve while the larger cowbird offspring consume most of the food brought by the parents.
The preferred habitat of the brown-headed cowbird is open agricultural areas.
The results of a study into the effects of deforestation on cowbird parasitism of four different host species are shown below.



Robinson et al. 1995

(a) State the effect of deforestation on cowbird parasitism. (1)

(b) Compare the effect of deforestation on cowbird parasitism of the worm-eating warbler and the Kentucky warbler. (2)

(c) Determine the percentage of worm-eating warbler nests parasitized by cowbirds at a level of 60% deforestation (1)

(d) Suggest reasons for the relationship between deforestation and cowbird parasitism. (2)

(*Total 6 marks*)

2 **(a)** Outline the use of Simpson's diversity index. (3)

(b) Explain the use of biotic indices and indicator species. (6)

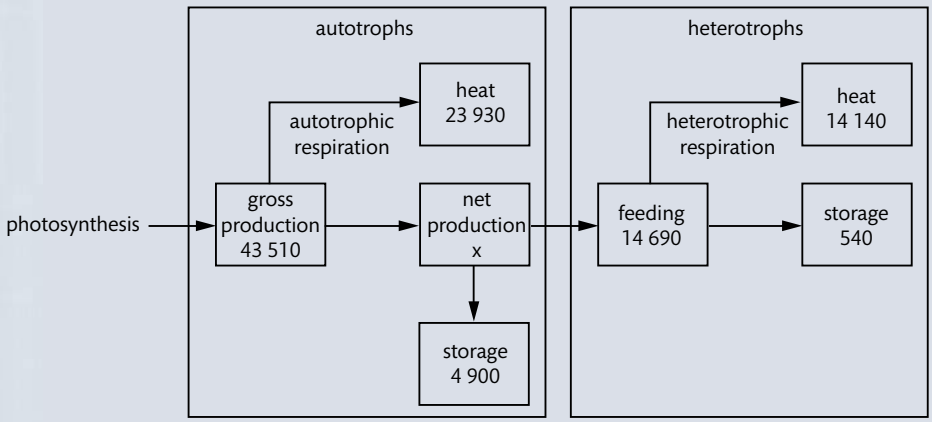
(*Total 9 marks*)

3 **(a)** Draw a labelled diagram of the nitrogen cycle. (3)

(b) State **two** fuels that can be produced from biomass. (2)

(*Total 5 marks*)

4 The energy flow diagram below for a temperate ecosystem has been divided into two parts. One part shows autotrophic use of energy and the other shows the heterotrophic use of energy. All values are kJ m^{-2} yr^{-1}.



(a) Calculate the net production of the autotrophs. (1)

(b) (i) Compare the percentage of heat lost through respiration by the autotrophs with the heterotrophs. (1)

(ii) Most of the heterotrophs are animals. Suggest **one** reason for the difference in heat losses between the autotrophs and animal heterotrophs. (1)

The heterotrophic community can be divided into food webs based upon decomposers and food webs based upon herbivores. It has been shown that of the energy consumed by the heterotrophs, 99% is consumed by the decomposer food webs.

(c) State the importance of decomposers in an ecosystem. (1)

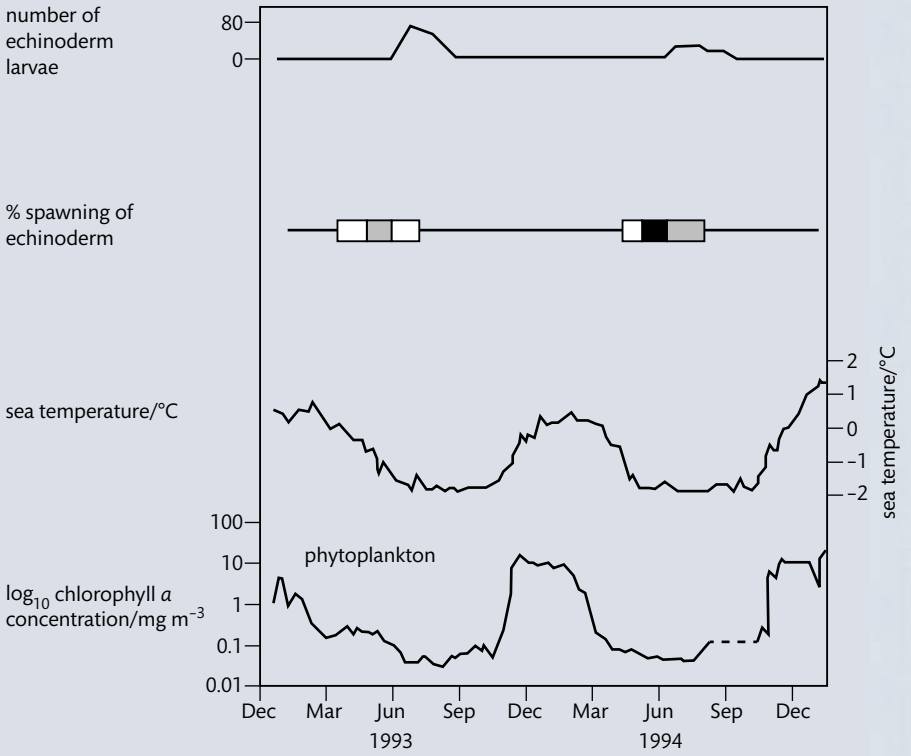
(d) Deduce the long-term effects of sustained pollution that kills decomposers on autotrophic productivity (2)

(*Total 6 marks*)

5 Sea-water temperature has an effect on the spawning (release of eggs) of echinoderms living in Antarctic waters. Echinoderm larvae feed on phytoplankton. In this investigation, the spawning of echinoderms and its effect on phytoplankton was studied.
In the figure below, the top line indicates the number of larvae caught (per 5000 l of seawater). The shaded bars below show when spawning occurred in echinoderms.

= 0% to 25%
= 25% to 75%
= 75% to 100%

The concentration of chlorophyll gives an indication of the concentration of phytoplankton.
Note: the seasons in the Antarctic are reversed from those in the northern hemisphere.



Adapted from Stanwell-Smith and Peck 1998

(a) State the trophic level of echinoderm larvae. (1)

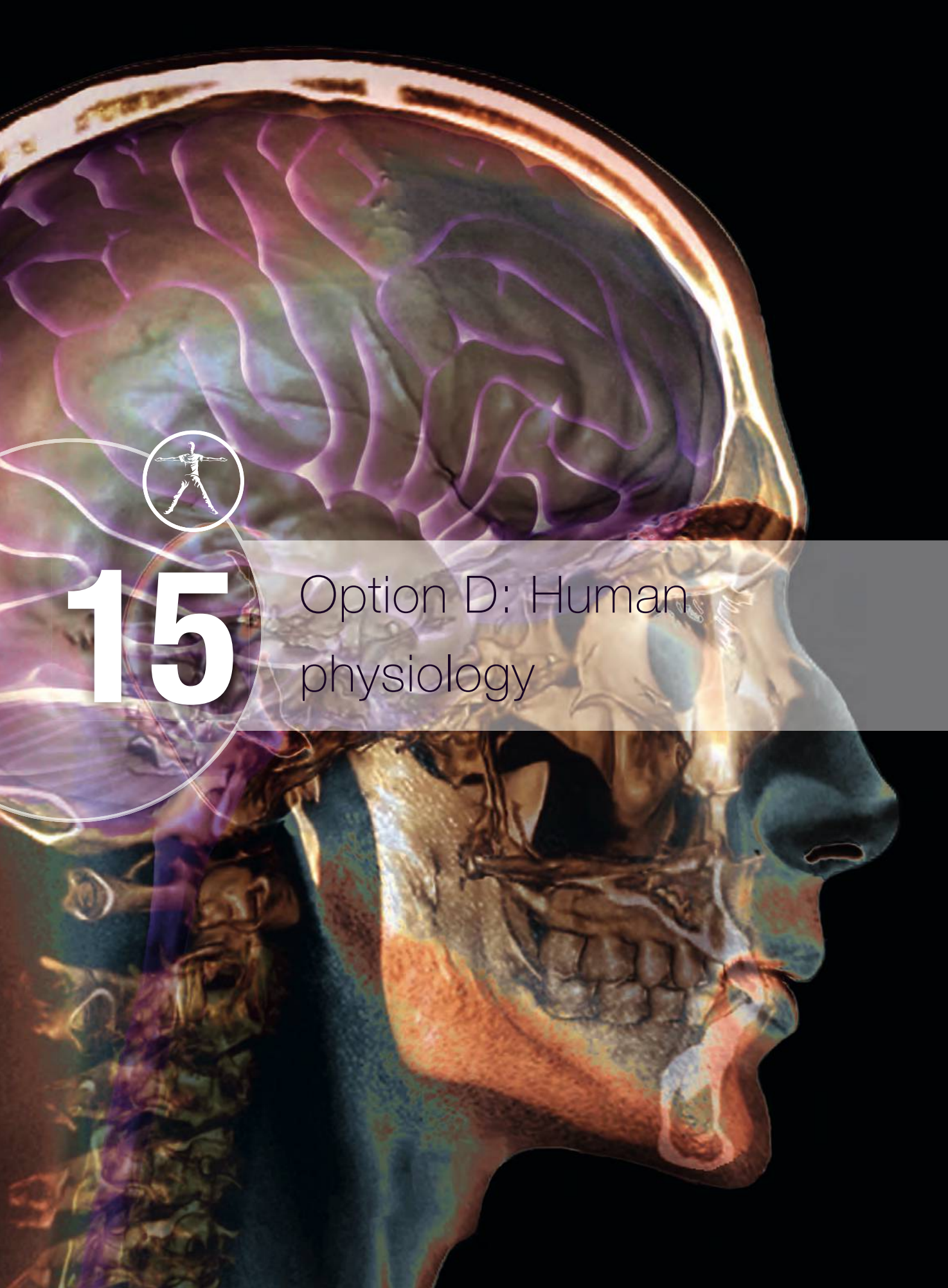
(b) Identify the period during which the spawning of echinoderm lies between 25% and 75%. (1)

(c) Explain the relationship between the seasons and the concentration of phytoplankton. (2)

(d) (i) Outline the effect of sea water temperature on echinoderm larvae numbers. (2)

(ii) Using the data in the figure, predict the effect of global warming on echinoderm larvae numbers. (2)

(*Total 8 marks*)



15 Option D: Human physiology

Coloured composite image of a magnetic resonance imaging (MRI) scan of the brain, and a three-dimensional (3-D) computed tomography (CT) scan of the head and neck, of a 35-year-old man.

Essential ideas

D.1 A balanced diet is essential to human health.

D.2 Digestion is controlled by nervous and hormonal mechanisms.

D.3 The chemical composition of the blood is regulated by the liver.

D.4 Internal and external factors influence heart function.

D.5 Hormones are not secreted at a uniform rate and exert their effect at low concentrations.

D.6 Red blood cells are vital in the transport of respiratory gases.

There is no end to what one can learn about human anatomy and physiology. In this chapter you will learn more in-depth detail about several of the systems of the body. Whether you want to pursue a career in medicine or just want to know more about the inner workings of human beings, this material can be fascinating.

D.1 Human nutrition

Understandings:

- Essential nutrients cannot be synthesized by the body, therefore they have to be included in the diet.
- Dietary minerals are essential chemical elements.
- Vitamins are chemically diverse carbon compounds that cannot be synthesized by the body.
- Some fatty acids and some amino acids are essential.
- Lack of essential amino acids affects the production of proteins.
- Malnutrition may be caused by a deficiency, imbalance, or excess of nutrients in the diet.
- Appetite is controlled by a centre in the hypothalamus.
- Overweight individuals are more likely to suffer hypertension and type II diabetes.
- Starvation can lead to breakdown of body tissue.

Applications and skills:

- Application: Production of ascorbic acid by some mammals, but not others that need a dietary supply.
- Application: Cause and treatment of phenylketonuria (PKU).
- Application: Lack of vitamin D or calcium can affect bone mineralization and cause rickets or osteomalacia.
- Application: Breakdown of heart muscle due to anorexia.
- Application: Cholesterol in blood as an indicator of the risk of coronary heart disease.
- Skill: Determination of the energy content of food by combustion.
- Skill: Use of databases of nutritional content of foods and software to calculate intakes of essential nutrients from a daily diet.

NATURE OF SCIENCE

Falsification of theories with one theory being superseded by another: scurvy was thought to be specific to humans, because attempts to induce the symptoms in laboratory rats and mice were entirely unsuccessful.

Essential nutrients: what are they?

A nutrient is a chemical substance found in foods and used in the human body. Nutrients can be absorbed to give you energy, help strengthen your bones, or even prevent you from getting a disease. You may recall from Section 2.1 that a handful of types of organic molecule make up all living organisms. Although some of these molecules, such as certain amino acids and lipids, can be synthesized by the human body, many cannot. Those that cannot be synthesized from other molecules, and thus must be a part of our diet, are called essential nutrients. They are:

- essential amino acids
- essential fatty acids
- minerals
- most vitamins.

Let's consider some examples of essential nutrients and the ramifications of a deficiency of those nutrients in the diet.

Dietary minerals: essential chemical elements

Minerals are the inorganic substances that living organisms need for a variety of purposes. Our world is full of minerals, but living organisms typically only need a very small intake of these elements to ensure good health. Each type of mineral has one or more specific role in making anatomical structures (e.g. calcium in bones) or a physiological role because it is incorporated into important molecules (e.g. iron within haemoglobin). These structures and molecules are typically 'long-lived' within the body, and thus the need for minerals is only for small amounts, but it is constant. The bones within our bodies require constant repair, requiring small amounts of calcium for that repair. Calcium ions are also used for other purposes within the body, and a small amount is always being lost and must be replaced. Red blood cells (erythrocytes) that contain haemoglobin have a cellular life span of only about 4 months. The components of erythrocytes are recycled within our liver, and much of the iron is recovered in order to produce more erythrocytes in the bone marrow. Some of the iron is inevitably lost, however, as the recycling is not 100% efficient. Females need more iron in their diet than males because the blood lost during menstruation leads to a loss of iron.

In many countries the food industries indicate the percentage of daily vitamins and minerals contained within a 'serving' of their products.

Many of the minerals required in our diet are known as electrolytes because they are easily dissolved in a fluid medium (e.g. blood, cytoplasm, and intercellular fluid) as charged ions. These charged ions include calcium (Ca^{2+}) and iron (Fe^{2+}), mentioned above, as well as sodium (Na^{+}), magnesium (Mg^{2+}), and chloride(Cl^{-}). Many of these electrolytes are particularly important in the mechanisms behind how we send action potentials along neurones, synaptic transmission between neurones, and muscle contraction. You may have experienced the pain involved in a 'muscle cramp' when an electrolyte imbalance occurs after strenuous exercise. This is just a small part of the story of minerals, as each has its own important role(s) within our physiology.

Vitamins: essential organic compounds

Unlike minerals, vitamins are organic (carbon-based) molecules. They are synthesized by living organisms, but many living organisms rely on an intake of vitamins from other organisms (especially plants, in the form of fruits and vegetables). Like minerals, the intake of vitamins needs only to be in small quantities, as vitamins are typically used to create relatively long-lived substances within the body.

A perfect example to illustrate the idea of an essential versus a non-essential vitamin is vitamin C (ascorbic acid) in humans. Vitamin C is not an essential vitamin in most animals, including the vast majority of vertebrates. However, it is essential for humans and thus must be a part of our diet. Failure to ingest enough vitamin C over an extended period of time results in a serious deficiency disease known as scurvy. Humans, some other primates, and guinea pigs are the only known animals where vitamin C is an essential vitamin.

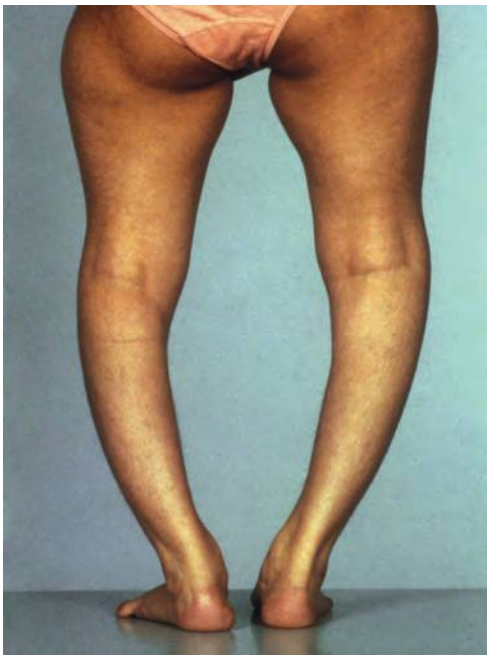
Vitamin C is produced from glucose in the kidney tissue in some animals, and in the liver in others. The synthesis of vitamin C from glucose requires four enzymes that are used in a step-by-step set of reactions. The gene coding for the fourth of these enzymes has been shown to be universally defective in all humans, thus making it essential that vitamin C is present in our diet.

Vitamin C should not be thought of as just a vitamin that prevents scurvy. Vitamin C is important in protection against infections, helping in wound healing, and in maintaining healthy gums, teeth, bones, and blood vessels.

To learn more about vitamin C, go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.1.

NATURE OF SCIENCE

Linus Pauling was an American chemist and biochemist who, in his book, *How to Live Longer and Feel Better* (1986), suggested that large doses of vitamin C would protect people against colds. This was a radical idea because vitamin C is normally regarded as a substance only useful in very small quantities. Pauling's ideas were not supported by conclusive results from clinical trials, so he was criticized by other scientists. Are suggestions given by established scientists more likely to receive acceptance than suggestions from lesser known researchers?



A person suffering from rickets with characteristic bowed legs as a result of improper bone plate growth. This develops in childhood and can be caused by vitamin D deficiency or calcium deficiency, or both.

Another essential component of the human diet is vitamin D. Vitamin D is an important nutrient for the proper formation of bones. Without a sufficient supply of vitamin D and/or the mineral calcium, it is possible to develop rickets, a disease that leads to deformities in the bones. Rickets develops in children when the bones near the growth plates (areas at the ends of developing bones) do not mineralize properly. This often leads to irregular, thick, and wide bone growth. The bone plates in adults are already fully formed, so rickets cannot develop. Children with rickets do not reach their optimal height during growth, and their legs are often bowed inwards or outwards at the knees. Even though adults cannot develop rickets, they can develop a similar condition called osteomalacia (pronounced os'te-o-mah-la´shah), which means soft bones. Osteomalacia is also the result of a deficiency in vitamin D or calcium.

The epidermis of human skin contains precursors that are able to synthesize vitamin D when stimulated by the ultraviolet rays of the Sun. Exposure to ultraviolet radiation has its own dangers, specifically sunburn and skin cancer, so everyone needs to balance the risks and rewards of obtaining vitamin D from the Sun.

The term precursor in biochemistry refers to a molecule that precedes another in a chemical reaction or metabolic pathway.

It is not possible to come up with a specific length of time that everyone should spend in sunlight to allow the synthesis of sufficient vitamin D. Factors such as latitude and sunlight intensity, seasonal variation, and genetic skin pigmentation have to be taken into consideration. However, typical suggestions range from about 5 to 30 minutes a day.

Fatty acids: two are essential

In Chapter 2 you learned that there are a variety of fatty acids that are components in triglycerides and phospholipids. If you recall, fatty acids all have a carboxyl functional group and a long hydrocarbon chain. Within that long hydrocarbon chain all of the carbon to carbon bonds may be single bonds (resulting in a saturated fatty acid), or one or more of the carbon to carbon bonds may be a double bond (resulting in an unsaturated fatty acid). The identity of the fatty acid is determined by its number of carbon atoms and the location(s) of the double bond(s). Two fatty acids are required in our diet because humans lack the enzymes to make these fatty acids from other fatty acids or precursors. These two fatty acids are omega-3 and omega-6. Both of these fatty acids are essential in the human diet and indicate that consuming fats is not necessarily bad for your health. The source and therefore the type of fat consumed is the key to good health.

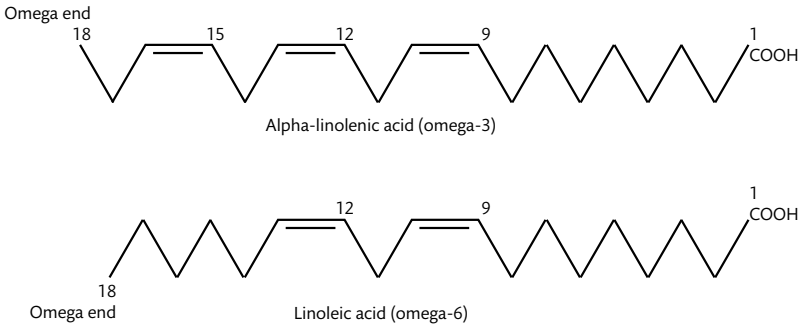


Figure 15.1 The two essential fatty acids shown in abbreviated form. Carbon number 1 is the carbon of the carboxyl group. Each angle change after that represents a carbon atom. Carbon atoms with double bonds are shown, and the first is numbered. Each carbon in the chain would have an appropriate number of hydrogens to make four bonds around each. The carbon on the far left of each structure is called the omega carbon. Counting from the omega carbon, you can easily see why these fatty acids are called omega-3 and omega-6, respectively. There is no reason to memorize these structures.

Cholesterol is a lipid substance needed in the body for a variety of reasons. Unfortunately, many people have levels of cholesterol circulating in their bloodstream that are excessive and can create problems within their blood vessels. Over time, as a condition called atherosclerosis develops, cholesterol can help form deposits called plaque on the inside of arteries. The inside of the artery slowly becomes smaller and smaller as the plaque continues to form. One of the more serious locations for this to occur is in the arteries that feed oxygenated blood directly into the heart muscle itself. These blood vessels are called the coronary arteries. The result is coronary heart disease, which can lead to a serious heart attack.

Amino acids: nine of 20 are essential

You would think it would be easy to specify the exact number of amino acids that are essential for humans. There is no doubt about nine of the 20: these nine are definitely essential, for everyone throughout their lives. After that it becomes a little less clear. For example, there are amino acids that are only essential for very young people, or for people who are suffering from a particular disease. Bear in mind what it means to be an 'essential' substance. Essential substances are no more important for our physiology than any other substances, but they are substances that cannot be synthesized from other molecules and thus must be a part of our diet. In the case of amino acids, a lack of one or more of the essential amino acids would mean that certain proteins could not be synthesized. The human body has no storage mechanisms for amino acids, so essential amino acids must be a part of your regular diet. People who live in cultures where their source(s) of protein comes from one or just a few food types can sometimes be in danger of a deficiency disease if their dominant protein source is low in one or more of the essential amino acids.

For example, some cultures are dependent on a single staple crop for much of their diet. One such staple crop is corn or maize. Corn is deficient in two essential amino acids, lysine and tryptophan. Populations that rely too much on maize as their primary source of protein can suffer from a variety of symptoms because of a low intake of these two amino acids. Researchers are developing an improved variety of maize that has increased levels of lysine and tryptophan.

Phenylketonuria (PKU)

Phenylketonuria (PKU) is a genetically inherited disease caused by a person's chemical inability to metabolize the amino acid phenylalanine. The inability to break down phenylalanine is a result of inheriting the mutated form of a gene that should be producing an enzyme (phenylalanine hydroxylase) that helps break down phenylalanine. Instead, phenylalanine builds up in tissues and the bloodstream. For a variety of biochemical reasons, excess phenylalanine can result in mental deficiency, behavioural problems, seizures, and other developmental problems. The allele for PKU is autosomal recessive (see Chapter 3 to remind yourself of these terms), and thus both parents must contribute an allele in order for the homozygous recessive condition to be expressed. Remember that both parents could be heterozygous individuals (carriers) who do not have PKU but do have a 25% chance of causing each of their children to have PKU. This gene defect is most common in European populations; it is much less common in Asians, Latinos, and Africans.

There is no cure for PKU, but there is a course of treatment that is effective as long as the disease is detected early. In countries where medical care is good, it is common for every newborn to be tested for PKU. If that test is positive, the treatment is based on a diet that limits proteins sources that are known to be high in phenylalanine. By simply limiting the intake of this one amino acid, the toxic levels characteristic of a 'normal' protein diet do not develop.

The incidence of PKU ranges between 1 in 2600 births in Turkey and 1 in 125 000 births in Japan.

A baby having a small amount of blood drawn from his or her heel to test for the possibility of PKU. This test is typically done very soon after birth so that a limited protein diet can be implemented as soon as possible if needed.

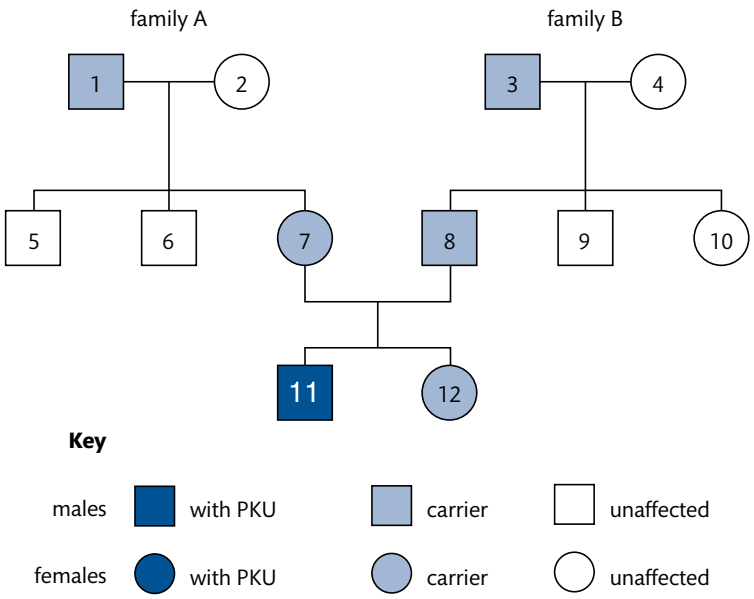


Figure 15.2 A pedigree showing the inheritance of PKU. Notice that the disease can be 'hidden' in families for several generations before manifesting itself when two carriers have children. The disease is not sex-linked, thus the male being shown with PKU was coincidental.

Eating and nutrition disorders

There are a variety of disorders involving food that can affect humans. Some of these are the result of a lack of sufficient healthy food, while others are behavioural and physiological disorders. All aspects of eating and nutritional disorders are heavily infuenced by a person's culture.

Appetite is controlled by the hypothalamus

Hunger is the body's way of expressing its need for food. Appetite is the desire to eat. It is quite possible to experience hunger and yet not feel the desire to eat (i.e. to be hungry but have no appetite), for example when you are sick. On the other hand, it is very common to not be hungry but see something that looks too good to resist.

At the end of a meal, when you have eaten a sufficient quantity of food, you have reached a state of satiety, and that is when most people stop eating. Although the mechanisms of appetite and satiety are quite complex and not fully understood, they seem to be a combination of feedback loops from the nervous system, the digestive system, and the endocrine (hormonal) system. For example, after a meal the pancreas releases hormones that reduce appetite. The question is, where do the feeling of hunger and the sensation of appetite originate in the body? To understand this, let's consider what happens when there is a problem with the system.

People who have medical complications that damage their hypothalamus (a part of the brain found at its base) can have severe appetite problems: some become very thin because of a loss of appetite, while others become very obese because of an insatiable appetite. From this evidence, it is clear that the hypothalamus plays an important role in regulating appetite. Although it has other functions as well, it can be said that the hypothalamus acts as your appetite control centre. During a meal, your stomach fills with food, expands, and stimulates cells of the vagus nerve. A signal is sent to the hypothalamus to stop eating. The intestines produce various hormones to send signals about hunger and satiety to the brain.

Anorexia is an eating disorder characterized by an obsession about body image, weight, and what foods to eat. Often sufferers of anorexia have an imagined 'ideal' body image that is far too underweight for good health. Sometimes the greatly restricted diet is accompanied by excessive exercise. The end result is not only a body that is far too thin, but a physiology that is in grave danger of collapsing because of a lack of essential nutrients. Even the heart muscle and internal valves can suffer damage that can be life threatening. If you know someone who appears to have the eating and exercise behaviours characteristic of anorexia, try to encourage him or her to get help because his or her life could be in danger.

In addition, the cells of adipose (fat) tissue produce a hormone called leptin that sends a message to the hypothalamus to suppress appetite. A person with more body fat produces more of this hormone, so that the brain knows there are adequate energy stores. If you fast, your level of leptin significantly decreases. But leptin is not the only hormone involved in the process of appetite; it would be an oversimplification to think that appetite was regulated solely by leptin, and other factors, such as compulsive eating and persuasive advertising, seem to be able to override leptin's effects.

The hypothalamus is found at the base of the brain as part of the brainstem. In addition to acting as the appetite control centre, the hypothalamus has a variety of other functions important to your physiology.

Consequences associated with being overweight

The perception of being underweight, normal, or overweight is highly biased by cultural and personal feelings about body shapes and expectations. A much better way to determine whether you have an appropriate weight is to calculate your body mass index (BMI). The BMI is a calculation of body mass that is corrected for height.

So, what are the health consequences of being overweight? Two of the more serious consequences are that people with high BMIs are much more likely to experience hypertension (high blood pressure) and develop type II diabetes.

Hypertension

There are many factors that can contribute to hypertension. Many of these factors are not controllable, such as age, ethnic origin, and family history. One of the factors that can be controlled is weight. There is a positive correlation between a higher BMI and hypertension. The more you weigh, the more blood you need to supply oxygen and nutrients to your cells. As the volume of blood circulated through your blood vessels increases, so does the pressure on the internal walls of your arteries.

Type II diabetes

Like hypertension, there are several factors that may contribute to the development of type II diabetes. But the data show that there is a positive correlation between developing type II diabetes and the occurrence of obesity. Type II diabetes used to be commonly called adult-onset diabetes because it was much more common to develop the symptoms of this disease later in life. As obesity has become more common in children and teenagers, the incidence of type II diabetes for these age groups has also increased, and thus 'adult-onset diabetes' is now an inappropriate name. Type II diabetes is most often characterized by body cell resistance to the normal effect of insulin, as well as a decrease in insulin production. Insulin is the hormone that allows cells to remove glucose from the bloodstream. The result is that blood glucose levels remain abnormally high because cells are not receiving the glucose for normal metabolic activity. People with type II diabetes must control their carbohydrate intake carefully to keep their blood glucose level reasonably stable.

Nutrition problems and their consequences

Food quantity and quality is a serious problem in many areas of the world. Malnutrition is a term that can be used for any of three possibilities: deficiency, imbalance, or excess of nutrients.

Deficiencies

Earlier in this chapter we considered situations in which one particular essential substance was missing from the diet, such as vitamin C or vitamin D. Very specific diseases, such as scurvy and rickets, are the result. Sometimes deficiencies can exist for many essential substances, including the calories (energy) from foods. When there is a lack of calories in the diet, a person's body will first draw upon any reserves that it has for substances that are needed. Glycogen stored in the liver and muscles will be exhausted very quickly as a source of glucose. Body fat will then be used. Many people who live in areas of the world where the availability of any type of food is severely limited will have neither glycogen nor body fat to make use of. Instead they will have to make use of protein within their body as a source of energy. We do not have storage mechanisms for protein: we need to have a regular intake of protein that can be digested to provide the amino acids needed for our own protein synthesis. When energy is not available from ingested carbohydrates, lipids, or proteins, the body's metabolism begins a series of reactions that digests body tissues for energy. One of the primary tissue types that is used first is skeletal muscle. Typically a single muscle does not completely 'disappear' when it is being used as a source of energy: the muscle just gets thinner and is therefore far less useful. When human beings are in the late stages of starvation they may be described as being 'just skin and bones'. The reason for this is that the skeletal muscle has become so thin it appears to be non-existent.

Weak muscle development in children because of poor nutrition. When the body has to 'choose' between energy needs and muscle development, energy needs become the priority for staying alive.

Imbalance

In areas of the world where there is a single staple crop providing most of the nutritional needs for a population, there can be an imbalance of nutrients in the population's diet. Depending on the species of staple crop being grown, this

situation can lead to an overall imbalance of too many carbohydrates or a more specific deficiency of one or more essential nutrients. Even in areas of the world where excellent sources of nutrition are available, an individual's own choice of what is in his or her diet can lead to serious nutritional imbalances. The flourishing fast-food industry is a testament to how many people choose acquired tastes over good nutrition.

Excess of nutrients

An excess of nutrients leads to obesity. Back in 2005, the World Health Organization's Obesity Task Force estimated that 400 million people were obese and 1.6 billion were overweight. The World Health Organization defines overweight and obesity as abnormal or excessive fat accumulation that may impair health. The degree of fat accumulation affects a person's body mass index (BMI) and determines whether someone is obese, overweight, or neither. You can review information on BMI in Section 2.3. The numbers of people overweight and obese have continued to increase in the last few decades. The causes for these ever-growing numbers are complex but the most obvious culprits are:

- change in the types and quantities of food people eat
- change in the amount of physical activity people do on a daily basis.

Just a few generations ago, most people in the world lived on farms. A family's daily routine involved a significant amount of physical activity to care for the crops and animals. Today, a migration towards urban centres has greatly reduced the amount of daily physical activity. In addition, the amount of time people devote to procuring and preparing their own food has dramatically decreased. The result is often low-nutrition, high-calorie choices being made from the many ready-to-eat food products available today.

Exercises

1. List four essential nutrients.
2. What is the fundamental difference between an essential nutrient and a non-essential nutrient?
3. For a long time, scurvy was thought to be unique to humans, as scientists could not replicate the symptoms of scurvy in rats and mice, even when these animals were denied vitamin C for a long period of time. Why did these experiments fail to produce symptoms of scurvy?
4. Why is rickets (a disease cause by insufficient intake of vitamin D) unique to children?

To learn more about essential fatty acids and the Linus Pauling Institute, and about essential amino acids, go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.1.

D.2 Digestion

Understandings:

- Nervous and hormonal mechanisms control the secretion of digestive juices.
- Exocrine glands secrete to the surface of the body or the lumen of the gut.
- The volume and content of gastric secretions are controlled by nervous and hormonal mechanisms.
- Acid conditions in the stomach favour some hydrolysis reactions and help to control pathogens in ingested food.
- The structure of cells of the epithelium of the villi is adapted to the absorption of food.
- The rate of transit of materials through the large intestine is positively correlated with their fibre content.
- Materials not absorbed are egested.

NATURE OF SCIENCE

Serendipity and scientific discoveries: the role of gastric acid in digestion was established by William Beaumont while observing the process of digestion in an open wound caused by gunshot.

Applications and skills:

- Application: The reduction of stomach acid secretion by proton pump inhibitor drugs.
- Application: Dehydration due to cholera toxin.
- Application: *Helicobacter pylori* infection as a cause of stomach ulcers.
- Skill: Identification of exocrine gland cells that secrete digestive juices and villus epithelium cells that absorb digested foods from electron micrographs.

Guidance

- *Adaptations of villus epithelial cells include microvilli and mitochondria.*

Exocrine secretions are fundamental to the digestive process

Exocrine glands are glands that produce a secretion that is useful in a specific location in the body and are taken to that location by a duct. Exocrine gland ducts lead to two general locations of the body. One location is the surface of the body. Examples of this type of secretion to the surface of the body are tears (lacrimal fluid) secreted from lacrimal glands and carried through ducts to the surface of the eye, perspiration produced by sweat glands and taken to the skin surface by small ducts, and milk produced by the mammary glands and taken through ducts to the nipple opening in lactating mothers. The second general location is the interior (lumen) of some part of the alimentary canal (gut). The secretions that fall into this second category are fluids that are necessary for digestion. All of these are needed at specific locations in the alimentary canal. Table 15.1 summarizes some of the more important digestive exocrine secretions.

Table 15.1 Important digestive secretions

Exocrine secretion	Exocrine gland	Ducts lead to	Function of secretion
Saliva	Salivary glands	Mouth	Moistens food; contains the enzyme amylase
Gastric juice	Three cell types found in pits in the stomach wall	Interior of the stomach	A mucus protects the stomach; hydrochloric acid (HCl) denatures proteins; pepsin is an enzyme
Pancreatic juice	Pancreatic cells	Duodenum	Trypsin, lipase, and amylase are all enzymes; a bicarbonate solution helps neutralize partially digested food entering from the stomach
Bile	Liver	Gall bladder and duodenum	Emulsification of lipids

Gastric secretions and their control

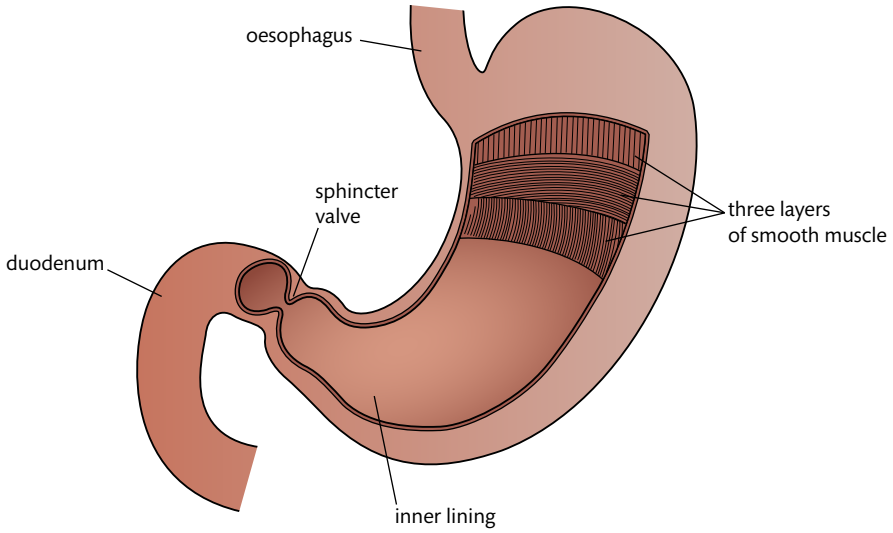


Figure 15.3 The term 'gastric' specifically refers to the stomach. Food enters the stomach from the tubular oesophagus. A valve located at the other end of the stomach remains closed for a period of time to allow gastric secretions to act upon the ingested food. In this sketch, you can see the three smooth muscle layers of the stomach that provide a churning action to mix the food thoroughly with the gastric juice.

As you learned in Section 6.1, the stomach is not only a 'holding place' for ingested food, but it is also the site where the early steps of digestion occur. In order to do this, some of the cells making up the inner lining of the stomach must be glandular and, as you have seen, they are exocrine glands. There are three types of glandular cells located in what are called pits (gastric pits) extending down into the inner lining of the stomach.

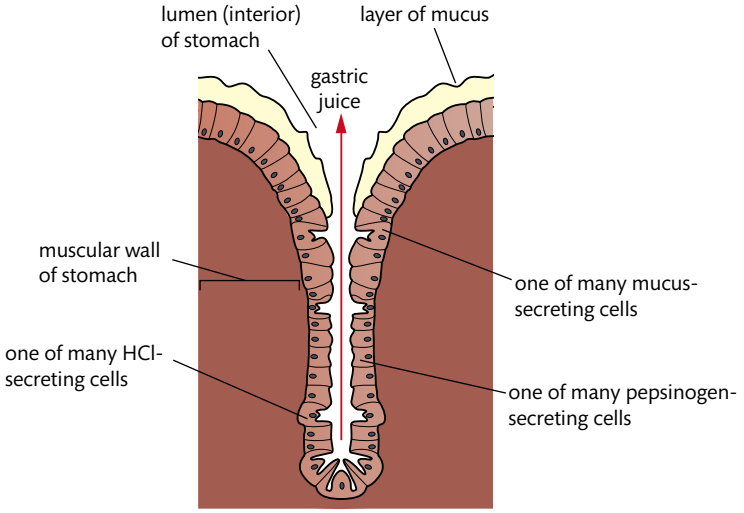


Figure 15.4 One of the many gastric pits located in the inner lining of the stomach. Each pit is shared by each of the glandular cell types creating and secreting one of the components of gastric juice (hydrochloric acid, pepsinogen, or mucus). Note the thin duct leading to the lumen of the stomach; the presence of this duct qualifies each of these pits as an exocrine gland.

Even before eating food, your stomach is being prepared for digestion. The thought, smell, sight, or taste of food results in autonomic nervous system impulses being sent to the medulla oblongata of your brainstem. The medulla oblongata responds using the parasympathetic division of the autonomic nervous system. Action potentials are sent by a cranial nerve called the vagus nerve directly to the stomach. The stomach then begins hydrochloric acid (HCl) and pepsinogen production and secretion into the

Late in the 20th century, researchers discovered a class of drugs that inhibit the production of acid by cells in the gastric pits of the stomach. Ever since then, these drugs have been available for people who suffer from conditions where the oesophagus becomes irritated by hydrochloric acid. This condition is generally known as acid reflux. In addition, some people develop ulcers, a condition where the stomach or duodenum has become irritated by acid as a result of a combination of thinned mucus and hydrochloric acid being in direct contact with the exposed tissue. By taking the acid-reducing drug(s) known as proton pump inhibitors (PPIs), the resulting decrease in acid production allows the irritated tissues to heal.

To learn more about protein pump inhibitors, go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.2.

cavity of the stomach. The same action potentials from the vagus nerve also stimulate endocrine cells in the lower portion of the stomach to secrete a hormone known as gastrin. Gastrin enters the blood and is carried to other cells elsewhere in the stomach, and results in even higher secretion of HCl and pepsinogen. When pepsinogen enters the cavity of the stomach and comes into contact with HCl, the pepsinogen converts into its active enzymatic form known as pepsin. Pepsin is one of many protease (protein-digesting) enzymes.

When food enters the stomach, the walls of the stomach become distended (expanded as a result of internal pressure). This results in an autonomic nervous system signal being sent by the vagus nerve to the medulla oblongata. The medulla oblongata then sends impulses back to the glandular cells of the stomach to continue (and increase) production of HCl and pepsinogen.

Finally, when a valve at the lower end of the stomach opens and releases the partially digested food (called chyme) into the duodenum of the small intestine, a set of signals terminates the secretion of acid and pepsinogen from the gastric pits. This includes production of a hormone called secretin that enters the blood and results in lowered gastric pit activity.

What is the role of HCl during the digestive process?

Remember that digestion is a chemical process that generally converts macromolecules (like proteins) into smaller 'absorbable size' molecules (like amino acids). When proteins enter the stomach, they are in their three-dimensional fibrous or globular molecular shapes characteristic of the secondary, tertiary, and quaternary shapes of this type of molecule (see Section 2.4). If you recall, there are many internal bonds holding proteins in these three-dimensional shapes, including numerous hydrogen and ionic bonds between non-adjacent amino acids. Also remember that one of the environmental factors that denatures proteins is pH conditions outside a protein's norm (see Section 2.4). In the highly acidic environment of the stomach, most proteins are far outside their normal pH range, and thus become denatured. This means that many of the hydrogen and ionic bonds that help shape the molecule become broken. The result is that the protein 'opens up' and digestive (hydrolytic) enzymes are able to more easily access the peptide bonds between adjacent amino acids.

Pepsinogen is one of the enzymes that benefits from the activity of HCl. When pepsinogen is first secreted from the gastric pits into the cavity of the stomach, it is in an inactive form. When the pepsinogen comes into contact with the HCl, it undergoes a molecular modification that activates the enzyme. At that point the enzyme is called pepsin. The function of pepsin is to catalyse the hydrolysis of large polypeptide chains into smaller peptides. The smaller peptides will be acted on by other protein-digesting enzymes later in the digestive process. In addition to activating pepsin, the highly acidic environment of the stomach is the ideal pH for the enzymatic activity of pepsin.

One final function of HCl in the stomach is to help control the ingestion of some pathogens. Many foods contain bacteria and fungi, and the vast majority of these are not harmful within the alimentary canal. A small percentage are harmful (pathogenic), and the highly acidic environment of the stomach helps to kill many of these before releasing the chyme into the small intestine.

A cow fitted with a fistula for observing and taking fluid samples from the rumen (one of its stomachs). The fistula is a surgically implanted 'window' that does not harm the animal.

What causes stomach ulcers?

The answers to scientific questions sometimes change. Can anything live in the highly acidic environment of our stomach? Until fairly recently, the answer to that question was thought to be no. The fluid in the stomach can be as acidic as pH 2. The consensus among scientists was that no living organism could survive such a harshly acidic environment.

In the early 1980s, two researchers isolated living bacterial cells (*Helicobacter pylori*) from the stomach lining of patients suffering from stomach ulcers. The conventional wisdom at that time was that stomach ulcers were caused by excess production of HCl, perhaps brought on by stress. Here is a summary of the more recent scientific information concerning stomach ulcers and gastritis (inflammation of the stomach).

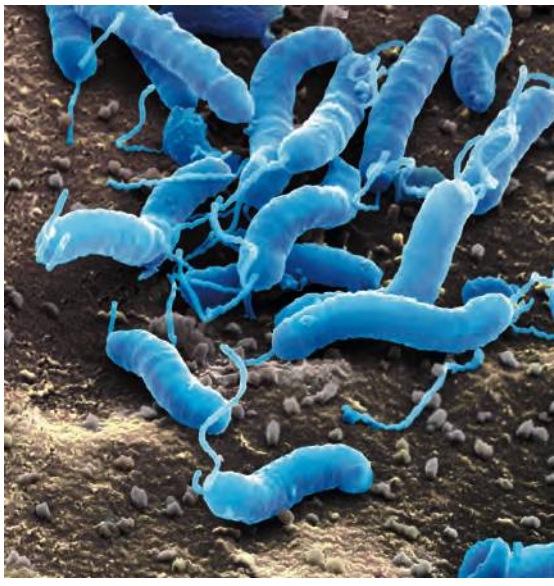
- *H. pylori* survives when introduced into the stomach, probably by burrowing beneath the mucus layer and infecting stomach lining cells.
- *H. pylori* employs the enzyme urease to create ammonia, and this helps to neutralize stomach acid.
- *H. pylori* infection of the stomach lining leads to gastritis and stomach ulcers.
- Patients treated with a selected range of antibiotics respond well to treatment.
- Patients with gastritis (and therefore infected with *H. pylori*) for many years (20–30 years, for example) are much more prone to stomach cancer than the general population.
- *H. pylori* infection may well be the most common bacterial infection in the world, as it is estimated that more than 3 billion people are infected.

NATURE OF SCIENCE

In 1822, an American physician by the name of William Beaumont saved the life of a Canadian trapper who had suffered a shotgun wound at close range. The wound left a permanent hole in the man's abdomen and stomach wall, allowing Beaumont to make observations and take samples of the digestive process.

TOK

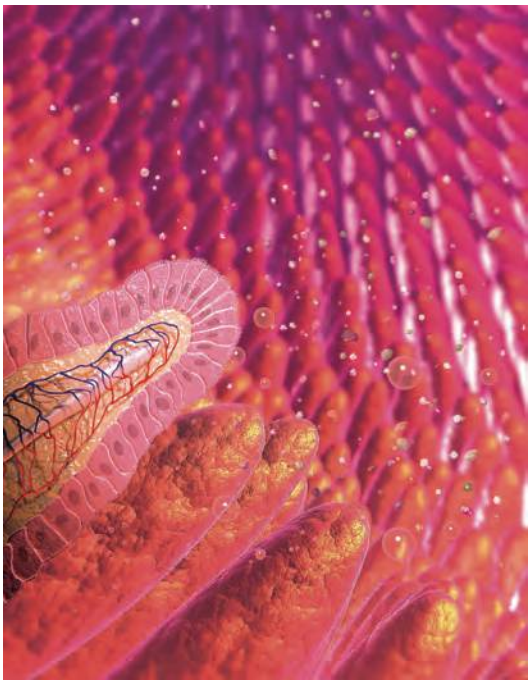
Hopefully, you have begun to view all sciences as a process, or perhaps a way of 'knowing'. Anyone who looks at a science topic as only a set of things to memorize is missing the much bigger and more important picture. Please don't memorize this.



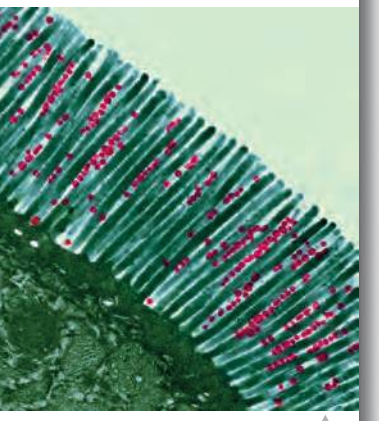
A scanning electron micrograph (SEM) of *H. pylori* in the stomach. This bacterial infection can result in gastritis, stomach ulcers, and possibly even stomach cancer if the infection persists for many years.

Adaptations of villi epithelial cells for efficient absorption

Digested molecules must pass through epithelial villi cells, and are absorbed into either a capillary or a lacteal on the interior of each villus. The surface of each villus cell that faces into the lumen (cavity) of the small intestine has many microscopic finger-like projections known as microvilli. The function of microvilli, like that of villi, is to increase greatly the surface area for absorption (compared with what it would be if the interior of the intestine was smooth).



An artist's representation of villi in the small intestine. Each villus contains a capillary bed and lacteal for the absorption of nutrients. The villi epithelial cells are the cells in contact with the nutrients inside the lumen of the intestine. Nutrients must pass through these cells in order to get to the capillaries and lacteal.



False-colour transmission electron micrograph (TEM) showing the microvilli of an epithelial cell extending into the intestinal lumen.

Some of the molecules absorbed through the plasma membranes of the villi are absorbed using an active transport mechanism. The requirement of active transport mechanisms for adenosine triphosphate (ATP) partly explains why the epithelial villi cells contain mitochondria. In addition, near the plasma membrane surface, pinocytotic vesicles are often visible. Pinocytosis is another active transport mechanism often used to absorb molecules from the lumen of the intestine into the interior of the villi cells, and also requires ATP from the mitochondria. Most cells in the body are surrounded by intercellular (interstitial) fluid. Even cells that make up the outer boundary of an organ typically allow molecules to move between cells. This would be an unacceptable situation for epithelial cells that make up villi. If intercellular fluid and dissolved molecules moved between adjoining cells, nutrients would have no selective barrier to pass through. It is the movement of digested molecules through the selectively permeable membrane of the villi epithelial cells that guarantees that the molecules have completed the process of enzymatic digestion. To this end, epithelial cells of villi are sealed to each other by membrane-to-membrane protein 'seals' called tight junctions (see Figure 15.5). The two cell membranes share some membrane proteins. This results in the two membranes being held so tightly together that most molecules cannot pass between them and must be transported first into and then out of the epithelial cells lining each villus.

On the side of the villi epithelial cell opposite where the microvilli are located (closer to the capillary bed), the plasma membrane has infoldings (invaginations) in order to increase the surface area for transport out of the epithelial cell. These invaginations are called the basal labyrinth and operate in the opposite direction but have a similar function as the microvilli.

When studying, ask yourself how well you know something. A general rule of thumb is, if you know it well enough to explain to someone else, then you know it well enough.

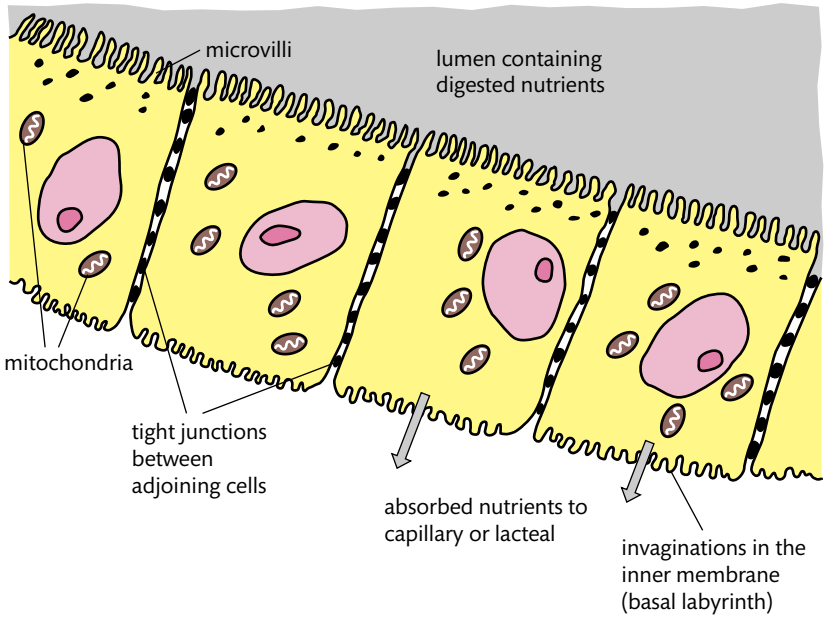
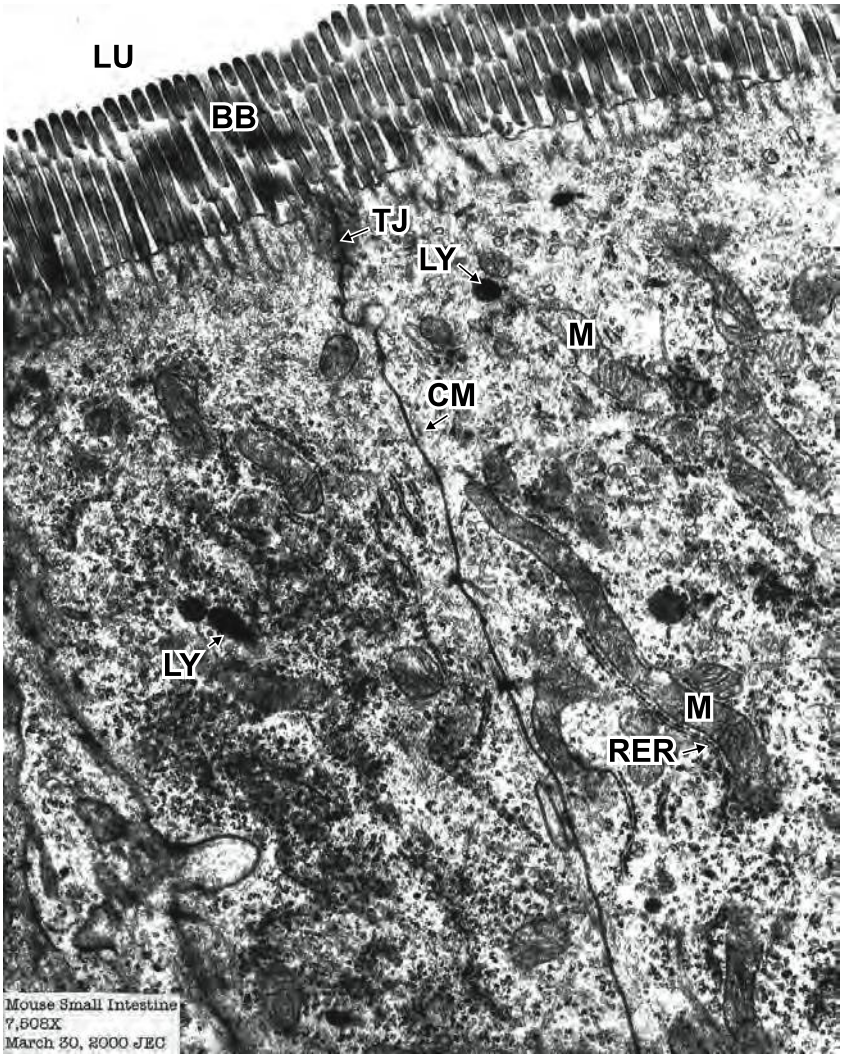


Figure 15.5 Individual epithelial cells of a villus. Digested molecules must pass through these cells in order to reach a capillary bed or lacteal.

CHALLENGE YOURSELF

1 See if you can identify the epithelial cell adaptations described in the previous section on the electron micrograph shown on the left. The photo shows two partial epithelial cells. The photograph does not show the 'lower' portion of each of the cells where the basal labyrinth is located. A key for the letter abbreviations is provided in the caption.



LU, the lumen of the small intestine (nutrients to be absorbed are found here); BB, brush border (the collective name for all the microvilli); TJ, tight junction; M, mitochondrion; RER, rough endoplasmic reticulum; LY, lysosome (organelles that contain digestive (hydrolytic) enzymes for use within the cell); CM, cell membrane.

To learn more about cholera go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.2.

Cholera is a disease caused by the bacterium *Vibrio cholera*; more specifically it is caused by the toxin secreted by *V. cholera*. The toxin results in a severe diarrhoea that leads to dehydration and is frequently fatal. Cholera is spread by drinking water or food contaminated with the bacterium. At one time cholera outbreaks occurred in almost every area of the world. Today, areas that have modern sewage processing and drinking water treatment rarely have problems with cholera. However, outbreaks still occur regularly in some areas of the world, and specifically in areas that have suffered catastrophic disasters such as tsunamis or major earthquakes.

The importance of fibre in the diet

Materials that are not absorbed are egested (become part of faecal matter).

Almost all absorption of nutrients occurs in the small intestine. However, some ingested substances will never be digested and thus have no chance of being absorbed into the bloodstream. These substances continue into the large intestine and become a part of the solid waste (faeces). These substances include:

- cellulose, from the cell walls of ingested plant material
- lignin, another component of plant cell walls
- bile pigments, from bile, which give the characteristic colour to faeces
- bacteria, because a few survive the low pH in stomach and become a constantly regenerating population of billions of mutualistic inhabitants of our digestive tract.

The human large intestine is populated with billions of bacteria. These bacteria are mutualistic because they provide us with vitamin K and a normal intestinal environment, while we provide the bacteria with undigested food from the small intestine.

How many times have you been told to 'eat up your vegetables'? Besides being a good source of vitamins and minerals, vegetables are an important source of fibre, although they are not the only fibre-rich foods. Fresh fruit and salads are also good sources of fibre.

Fibre, also referred to as dietary fibre (or, more informally, roughage), is composed mostly of the cellulose and lignin in plant material (see the list above). It helps the human digestive system function better by providing bulk. In order for peristalsis (smooth muscle contractions that propel material through the alimentary canal) to function optimally, the muscles that push 'food' along the intestines need to have a sufficient volume of material to apply pressure to. Not surprisingly, the rate of movement of material through the large intestine has a positive correlation with fibre content.

There is a positive correlation between the amount of fibre in a person's diet and the rate of movement of material through his or her large intestine.

High-fibre diets also help people manage their body mass better. It is easier to lose excess weight with a diet that includes fruits and vegetables, in part because the fibre fills up the stomach, giving a feeling of satiety without introducing excess energy. A common criticism of modern diets, especially in industrialized countries, is that they do not contain enough fibre. One recommendation is to eat at least five servings of fruit or vegetables each day.

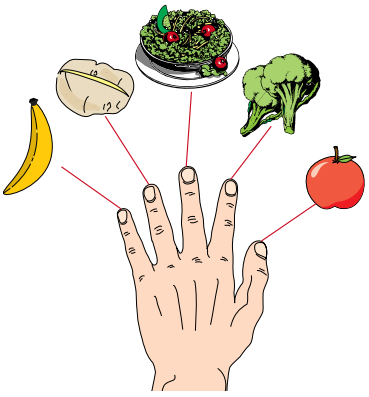


Figure 15.6 To help you remember to eat at least five serving of fruits and vegetables every day, count them on your fingers.

Exercises

5 What are the three components of gastric juice? Summarize the function of each.

6 You are sitting at the dining room table with your parents. They both mention that they are worried about getting a stomach ulcer because of the stress they are under at work. What would you tell them?

7 What are some of the adaptations of epithelial villi cells that allow them to be efficient at absorbing digested nutrients and passing those nutrients on to the bloodstream or lymphatic system?

8 Explain the general function of an exocrine gland.

D.3 Functions of the liver

Understandings:

- The liver removes toxins from the blood and detoxifies them.
- Components of red blood cells are recycled by the liver.
- The breakdown of erythrocytes starts with phagocytosis of red blood cells by Kupffer cells.
- Iron is carried to the bone marrow to produce haemoglobin in new red blood cells.
- Surplus cholesterol is converted to bile salts.
- Endoplasmic reticulum and Golgi apparatus in hepatocytes produce plasma proteins.
- The liver intercepts blood from the gut to regulate nutrient levels.
- Some nutrients in excess can be stored in the liver.

Applications and skills:

- Application: Causes and consequences of jaundice.
- Application: Dual blood supply to the liver and differences between sinusoids and capillaries.

NATURE OF SCIENCE

Educating the public on scientific claims: scientific studies have shown that high-density lipoprotein could be considered 'good' cholesterol.

Circulation of blood to and from the liver

The liver receives blood from two major blood vessels, and is drained by one (see Figure 15.7). The hepatic artery is a branch of the aorta and carries oxygenated blood to the liver tissues. The hepatic portal vein is the other blood vessel supplying blood to the liver. These two blood vessels carry blood into the capillaries of the liver, called sinusoids. All sinusoids are then drained by the hepatic vein, which is the sole blood vessel taking blood away from the liver.

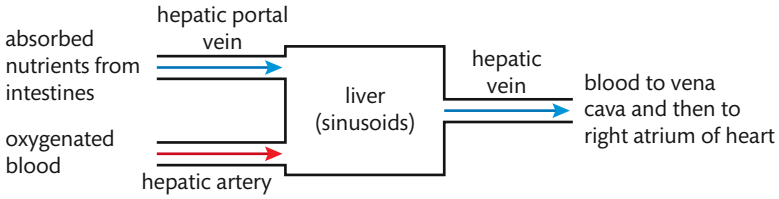


Figure 15.7 A schematic showing the blood circulation pattern to and from the liver.

The hepatic portal vein receives blood from the capillaries within all the villi of the small intestine. The blood within the hepatic portal vein varies in two ways from blood that normally arrives at an organ:

- it is low-pressure, deoxygenated blood because it has already been through a capillary bed
- it varies considerably in quantity of nutrients (especially glucose), depending on the types of food and the timing of ingestion, digestion, and absorption of food within the small intestine.

The blood within the hepatic vein is also low-pressure, deoxygenated blood, but it does not vary in nutrients as much as the blood within the hepatic portal vein. The stabilization of nutrients within the hepatic vein represents one of the major functions of the liver, specifically the storage of nutrients and the release of those nutrients when needed.

A portal system of circulation (like the hepatic portal system described here) is when blood travels through two capillary beds before returning to the heart to be re-pumped.

Sinusoids are the capillaries of the liver

The function of the liver is to remove some substances from the blood and add others to it. This removal or addition of a variety of substances is the job of the hepatocytes (liver cells). Oxygen-rich blood from the hepatic artery and (sometimes) nutrient-rich blood from the hepatic portal vein both flow into sinusoids of the liver. Sinusoids are where exchanges occur between the blood and the hepatocytes (see Figure 15.8).

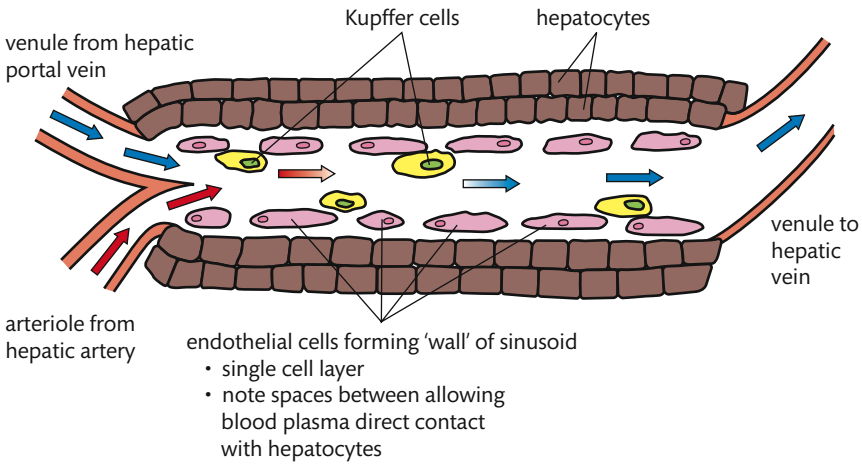


Figure 15.8 Sinusoids are the capillary beds of the liver, but their structure and action are different from capillary beds found elsewhere in the body.

Sinusoids differ from a typical capillary bed in the following ways:

- sinusoids are wider than capillaries
- sinusoids are lined by endothelial cells with gaps between them
- these gaps allow large molecules like proteins to be exchanged between hepatocytes and the bloodstream
- hepatocytes are in direct contact with blood components, making all exchanges with the bloodstream more efficient
- sinusoids contain Kupffer cells that help break down haemoglobin released from 'older' erythrocytes for recycling cell components
- sinusoids receive a mixture of oxygenated blood (from hepatic artery branches) and nutrient-rich blood (from hepatic portal vein branches), and this mixture eventually drains into small branches of the hepatic vein.

The liver does not extract all excess glucose, toxins, etc., on a single pass of the blood through the liver sinusoids. The hepatocytes act on the chemicals within the blood many times as the blood makes a continuous circuit through the liver.

The liver removes toxins from the blood

A typical human being ingests an amazing number of toxic substances every day. These toxins come in the form of pesticides and herbicides added to food produce, food preservatives, food flavour 'enhancers', medications, and alcohol, to name just a few. The reason we do not think of many of these substances as being toxic is because our bodies have efficient mechanisms in place to process and eliminate them. The liver contains two kinds of cells that are used in these processes.

1. Kupffer cells: these cells line the inside of sinusoids and use phagocytosis to remove old erythrocytes and bacteria from the blood. They are therefore phagocytic and contain many lysosomes. Kupffer cells are specialized leucocytes (white blood cells).
2. Hepatocytes: these are the most numerous cells in the liver, and are the most active in removing and processing chemical toxins from the blood. When blood

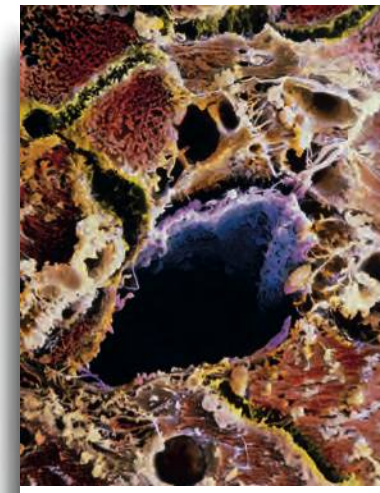
flows through the sinusoids, hepatocytes are bathed with the liquid (plasma) component of blood. They extract toxins from the plasma and begin a two-step process to eliminate the toxins. First, they chemically modify the toxin to make it less destructive, and second, they add chemical components that make the (now modified) toxin water soluble. The water-soluble modified substance can be added back into the blood in order to be eliminated by the kidneys as a component of urine.

Alcohol consumption damages liver cells over time

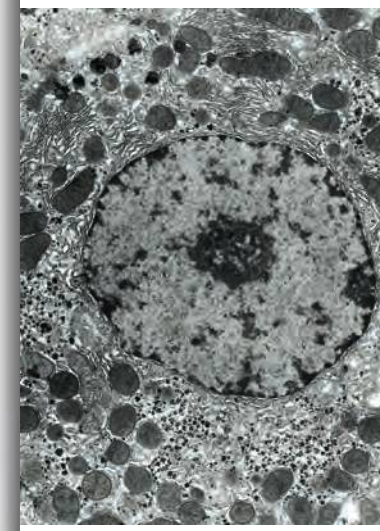
People who drink alcohol, especially often and in high volume, can expect liver damage. As is the case with useful nutrients, the hepatic portal vein brings absorbed alcohol to the liver first. Any alcohol not removed the first time is brought back through the liver sinusoids by the hepatic artery. Each time the blood passes through the liver, hepatocytes attempt to remove the alcohol from the bloodstream. Thus alcohol has a magnified effect on liver tissue compared with other tissues in the body. It has been shown that long-term alcohol abuse results in three primary effects on the liver.

- Cirrhosis: this is the scar tissue left when areas of hepatocytes, blood vessels, and ducts have been destroyed by exposure to alcohol. Areas of the liver showing cirrhosis no longer function.
- Fat accumulation: damaged areas of the liver will quite often build up fat in place of normal liver tissue.
- Inflammation: this is the swelling of damaged liver tissue as a result of alcohol exposure, sometimes referred to as alcoholic hepatitis.

The liver can repair itself if damage is not too severe, but long-term alcohol abuse can be fatal.



False-colour SEM of liver cells with cirrhosis. A sinusoid is visible (blue) surrounded by abnormal hepatocytes. Many fibres of connective tissue (light brown) have invaded the damaged area.



TEM of a section through a rat liver cell. At the centre is the nucleus, containing a single nucleolus. The dark ovoid objects spread throughout the cell are mitochondria surrounded by large numbers of endoplasmic reticulum. The small black dots are glycogen granules, the storage form of glucose.

Regulation of nutrients in the blood

Solutes that are dissolved in blood plasma vary a little in concentration, but each type of solute has a normal homeostatic range. Any concentration below or above this normal range creates problems in the body.

Let's consider glucose as an example. For most people, the glucose levels in blood are lowest in the morning and highest soon after a meal. When you digest a meal that is high in carbohydrates, such as starch, your hepatic portal vein will contain blood with a very high concentration of glucose. When this blood enters the sinusoids of your liver, some of the excess glucose is taken in by the surrounding hepatocytes and converted to the polysaccharide glycogen. This keeps the glucose level in the normal range. Stored glycogen can be seen as large vesicles or 'granules' in electron micrographs of hepatocytes.

Now imagine you have not eaten any carbohydrates for a long time. Your blood glucose levels decrease as cells use the glucose for cell respiration. To keep the glucose level in the normal range, the stored glycogen in the granules is reconverted to glucose and added into the bloodstream in the sinusoids.

The homeostatic mechanisms at work are regulated by the production of the hormones insulin and glucagon from the pancreas. When blood glucose levels are towards the upper end of the normal range, insulin is produced and this stimulates hepatocytes to take in and convert glucose to glycogen. When blood glucose levels

approach the lower end of the normal range, the pancreas produces glucagon and this hormone stimulates hepatocytes to convert glycogen back into glucose.

In addition to glycogen, other nutrients can be stored in the liver, as summarized in Table 15.2.

Table 15.2 Nutrients stored by the liver

Nutrient	Relevant information
Glycogen	A polysaccharide of glucose (sometimes called animal starch)
Iron	Iron is removed from haemoglobin, and later sent to bone marrow
Vitamin A	Associated with good vision
Vitamin D	Associated with healthy bone growth

The liver recycles components of erythrocytes and haemoglobin

Erythrocytes have a typical cellular life span of about 4 months. This means every erythrocyte needs to be replaced every 120 days or so by the blood cell-forming tissue of the bone marrow. This is necessary because erythrocytes are anucleate (they have no nucleus) and thus cannot undergo mitosis to form new blood cells, nor are they able to code for new proteins within the cell.

Kupffer cells are a type of leucocyte that resides in the sinusoids of the liver. Besides ingesting haemoglobin, they can also ingest cellular debris and bacteria within the bloodstream.

As erythrocytes approach the end of their approximately 120-day life, the cell membrane becomes weak and eventually ruptures. More often than not this occurs in the spleen or bone marrow, but it can happen anywhere in the bloodstream. The rupture leads to millions of haemoglobin molecules circulating in the bloodstream. As blood circulates through the sinusoids of the liver, these circulating haemoglobin molecules are ingested by Kupffer cells within the sinusoids. This ingestion is by phagocytosis because haemoglobin molecules are very large proteins.

Haemoglobin consists of four polypeptides (globins) and a non-protein molecular component at the centre of each globin called a haem group. At the centre of each haem group is an iron atom. Thus each haemoglobin consists of four globins, four haem groups and four iron atoms. It is within Kupffer cells that haemoglobin is disassembled into its component parts. The key events are summarized in the following bullet list and in Figure 15.9.

- The four globin proteins of each haemoglobin are hydrolysed into amino acids.
- The amino acids are released back into the bloodstream and become available to any body cell for protein synthesis.
- The iron atom is removed from each haem group. Some of this iron is stored within the liver and some is sent to bone marrow to be used in the production of new erythrocytes.
- Once iron has been removed from the haem group, what remains of the molecule is called bilirubin or bile pigment. This is absorbed by the nearby hepatocytes and becomes a key component of bile.

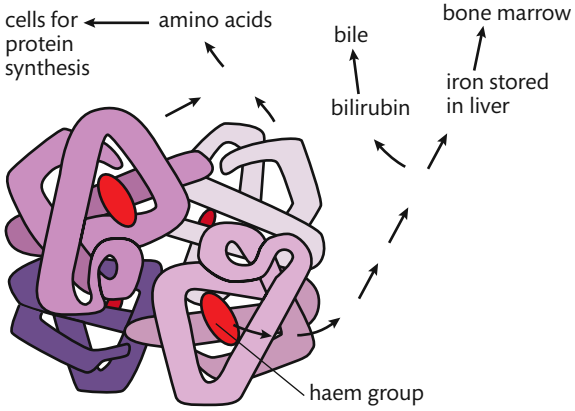


Figure 15.9 The molecular components of haemoglobin are recycled when erythrocytes die after about 4 months.

Hepatocytes produce and secrete bile and plasma proteins

One of the better-known functions of the liver is the production of bile. Bile is added to the duodenum when fatty foods are being digested in order to emulsify fats. Lipids (fats and oils) have a tendency to coalesce (clump) together because they are hydrophobic and thus not water soluble. This makes it difficult for the enzyme lipase to digest the lipids as very little surface area of the 'clump' is exposed. When bile is added into the duodenum, the resulting emulsification does not chemically change the lipids, but it does break up the coalesced clumps and increases the surface area for lipase to catalyse the digestion.

Hepatocytes within the liver produce bile by converting surplus cholesterol into a similar molecule known as a bile salt. These bile salts are added to bilirubin to make the substance bile. The bile salts are the emulsifying portion of bile.

Another well-documented function of hepatocytes is the production of many types of proteins that are added into the bloodstream. These are called plasma proteins because they circulate in the liquid portion of blood called blood plasma. There are many of these proteins produced by the liver, but two whose functions are documented elsewhere in this text are:

- albumin, which helps regulate blood osmotic pressure and acts as a carrier for bile salts and some other fat-soluble substances
- fibrinogen, which when converted to fibrin forms the mesh component of a blood clot.

Plasma proteins produced by the liver must also be secreted from hepatocytes. Thus the sequence of events is identical to that of any cell that produces and secretes a protein for use outside that cell.

1. DNA within the nucleus of a hepatocyte synthesizes messenger (m)RNA for a particular protein (transcription).
2. mRNA exits the nucleus through a nuclear pore.
3. mRNA finds a ribosome located on rough endoplasmic reticulum (ER).
4. Plasma protein is synthesized (translation).
5. Plasma protein is transported by a vesicle to the Golgi apparatus.
6. The Golgi apparatus possibly modifies the protein and surrounds the protein with another vesicle.

NATURE OF SCIENCE

Cholesterol in our diet has a bad reputation. To some degree and in some food types this reputation is deserved. However, many people don't understand that there are different kinds of cholesterol and that they are used for different purposes in the body. You might remember that we need one type of cholesterol in our cell membranes to provide flexibility. When you have your cholesterol checked with a blood test, there is one type of cholesterol/lipid that is considered to be 'good cholesterol'. It is abbreviated as HDL, standing for high-density lipoprotein.

About 95% of the bile salts that enter the small intestine are reabsorbed into the blood in the last portion of the small intestine. These bile salts enter the bloodstream and attach to the plasma protein called albumin. They are returned to the liver to be reincorporated into more bile.

7 The vesicle goes to the plasma membrane for exocytosis (secretion).
8 The plasma protein enters the blood plasma.

Causes and consequences of jaundice

Jaundice is a condition characterized by having too much bilirubin circulating in the bloodstream and thus within the body tissues. Bilirubin is a yellow pigment and so people with jaundice have a yellow tinge to their skin and a yellowing of the whites of their eyes. Bilirubin is formed when haemoglobin molecules are processed from dying erythrocytes. There are two main types of jaundice.

A newborn receiving phototherapy for infant jaundice. The light used emits blue–green wavelengths of the spectrum (not ultraviolet, as commonly believed).

1 Infant jaundice is found in newborns. It most typically occurs in babies who are born prematurely because their livers are not yet capable of fully processing the bilirubin into bile. Up to the point of birth, bilirubin is processed by the mother through the placenta. Soon after birth, a newborn may begin showing the yellowing symptoms of jaundice. Except in very serious cases, the most common treatment is exposure to the blue and green portion of the light spectrum. The blue–green light changes the shape and structure of bilirubin molecules, and they can then be eliminated in the baby's urine and stools. This gives the baby's liver time to mature for full processing of bilirubin into bile. The most severe consequence of untreated jaundice is a brain condition called acute bilirubin encephalopathy. Excessive bilirubin levels are toxic to brain cells, which is why newborns with symptoms of jaundice must be treated promptly.
2 Adult jaundice has many of the same symptoms and consequences as infant jaundice. The cause can always be traced back to liver function. The jaundice is therefore a symptom, and the underlying cause is whatever problem is leading to the liver not functioning properly. When the liver is not functioning properly, there are also likely to be many other symptoms.

Exercises

9 Briefly describe the blood supply into and out of the liver.
10 Explain why humans do not need excessive amounts of iron in their diet in order to make the millions of new erythrocytes that are formed each and every minute in the bone marrow.
11 Describe what would happen in the liver if a person was to go for an extended period of time without eating or exercised heavily for a long period of time.
12 Why does alcoholism lead to liver damage?

D.4 The heart

Understandings:

- Structure of cardiac muscle cells allows propagation of stimuli through the heart wall.
- Signals from the sinoatrial node that cause contraction cannot pass directly from atria to ventricles.
- There is a delay between the arrival and passing on of a stimulus at the atrioventricular node.
- This delay allows time for atrial systole before the atrioventricular valves close.
- Conducting fibres ensure coordinated contraction of the entire ventricle wall.
- Normal heart sounds are caused by the atrioventricular valves and semilunar valves closing, causing changes in blood flow.

Applications and skills:

- Application: Use of artificial pacemakers to regulate the heart rate.
- Application: Use of defibrillation to treat life-threatening cardiac conditions.
- Application: Causes and consequences of hypertension and thrombosis.
- Skill: Measurement and interpretation of the heart rate under different conditions.
- Skill: Interpretation of systolic and diastolic blood pressure measurements.
- Skill: Mapping of the cardiac cycle to a normal ECG trace.
- Skill: Analysis of epidemiological data relating to the incidence of coronary heart disease.

Guidance

- *Include branching and intercalated discs in structure of cardiac muscle.*

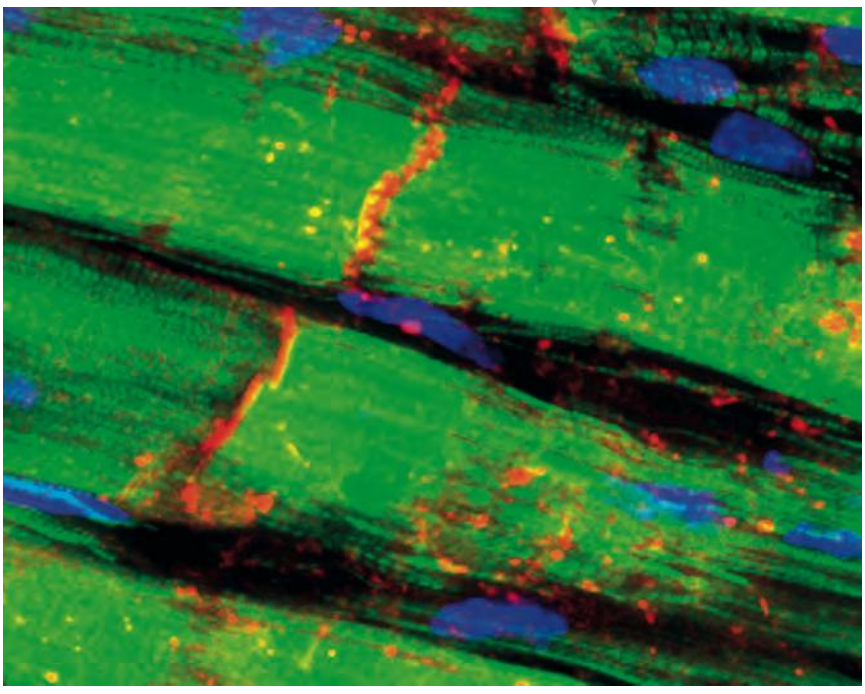
NATURE OF SCIENCE

Developments in scientific research followed improvements in apparatus or instrumentation: the invention of the stethoscope led to improved knowledge of the workings of the heart.

The heart is composed of cardiac muscle cells

Skeletal muscle is muscle that moves your bones to create various body motions. In skeletal muscle, many individual cells are fused together to make a fibre. The evidence for this is that the fibre contains many nuclei: it is said to be multinucleate. This arrangement makes it easier for the fibre to act as a single unit when contracting.

Cardiac muscle has some similarities with skeletal muscle, especially in the arrangement of the actin and myosin proteins in contracting units called sarcomeres. Cardiac muscle cells containing the sarcomeres remain as single cells joined together by interconnections called intercalated discs. These disc-shaped areas contain openings called gap junctions where cytoplasm from one cell freely passes to the next cell. This sharing of cytoplasm is what allows the cardiac muscle cells to pass an electrical signal so quickly from cell to cell. Without these gap junctions the impulse to begin a heart beat would spread too slowly through the muscle tissue to result in a unified event.



A light micrograph taken with fluorescent markers showing cardiac muscle cells. The light and dark green lines running horizontally are the sarcomeres. Two intercalated discs are shown (vertical orange lines). A variety of nuclei (blue) can be seen.

Intercalated discs contain structures known as gap junctions. Gap junctions are protein-lined channels that allow direct transmission of the nerve impulse from cell to cell so that cells contract in unison. Because of this, muscle cells are said to be 'electrically coupled'.

Cardiac muscle cells that are joined together by intercalated discs form fibrous units that repeatedly branch. The muscle tissue is dense with relatively large mitochondria, and has a very generous blood supply (it is said to be highly vascular). These adaptations help prevent cardiac muscle getting fatigued. The evolutionary design behind the repeated branches and individual cells joined by intercalated discs is based on getting the muscle cells to work together as a unit. All they need are signals to synchronize their contraction activity.

Illustration showing a small portion of cardiac muscle. Notice the branching between one area of muscle cells and another. There are several individual cardiac muscle cells shown, with two shown in section. The sections are shown with a portion of an intercalated disc cut in half. The sections also show sarcomeres and a large central nucleus (purple).

The cardiac cycle

The cardiac cycle is a series of events that we commonly refer to as one heart beat. More properly, one cardiac cycle is all the heart events that occur from the beginning of one heart beat to the beginning of the next heart beat. The frequency of the cardiac cycle is your heart rate, and is typically measured in beats per minute. If you have a resting heart rate of 72 beats min^{-1}, you are performing 72 cardiac cycles each minute.

When a chamber of the heart contracts, it is because the cardiac muscle of the chamber has received an electrical signal that has caused the muscle fibres of the chamber to contract. This causes an increase in pressure on the blood within the chamber, and the blood leaves the chamber through any available opening. This is called systole (pronounced sis-tol-ee). When a chamber is not undergoing systole, the cardiac muscle of the chamber is relaxed. This is called diastole (di-astol-ee). Both atria contract at the same time, therefore you can say that both undergo systole at the same time. Both ventricles also undergo systole simultaneously, just a little after the atrial systole.

Anatomical diagrams identify right and left sides as if it is your own body that is being shown. Most anatomical diagrams show a ventral view (from the front): so the left side of the body is on the right, and the right is on the left. Any diagram identified as a dorsal view (from the back) shows the right side on the right, and the left on the left.

Heart valves

Heart valves keep blood moving in a single direction. Each chamber of the heart has to have an opening to receive blood and another opening to allow blood to exit. When a chamber undergoes systole, it is imperative that the blood moves consistently in a single, useful direction (see Figure 15.10). The heart valves serve to prevent a backflow of blood.

The valves located between the atria and ventricles are called the atrioventricular valves (identified as right and left according to the side of the heart). The valves located where the blood exits the ventricles are called semilunar valves and are also identified as left and right (see Figure 15.10).

Each of the heart valves has at least one other name that you may well come across in books and texts. In order to avoid confusion, some of the more common synonyms (alternative names) are given in Table 15.3.

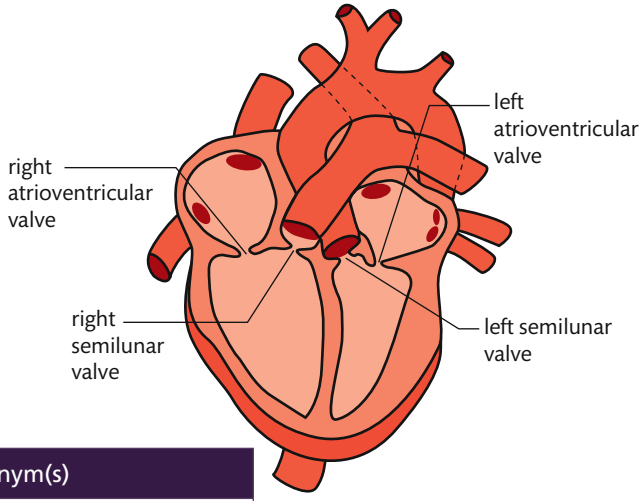


Figure 15.10 The location of the four heart valves.

Table 15.3 Different names for the valves of the heart

Heart valve	Synonym(s)
Right atrioventricular valve	Tricuspid valve
Left atrioventricular valve	Bicuspid valve, Mitral valve
Right semilunar valve	Pulmonary valve, Pulmonary semilunar valve
Left semilunar valve	Aortic valve, Aortic semilunar valve

You may have noticed that there are no valves where blood enters the atria. So what prevents blood from flowing back up into the vena cava and pulmonary veins when the atria undergo systole? The answer to this question is two-fold.

- Both the vena cava and pulmonary veins are veins, and thus have internal, passive flap valves characteristic of all veins. These are valves curved in the direction of blood flow that stay open as long as the blood is flowing in the proper direction within the vessel. If blood attempts to flow backwards in any vein, the passive flap valves use the force of the blood hitting the valve to close down and prevent blood from flowing in that direction.
- Atrial systole does not build up very much pressure. The muscular walls of the atria are very thin in comparison with the ventricles. Their force of contraction is slight in comparison with the ventricles. Thus the relatively low pressure exerted by the atria in combination with the passive flap valves within the supply veins means that no heart valve is necessary where the blood enters each atrium.

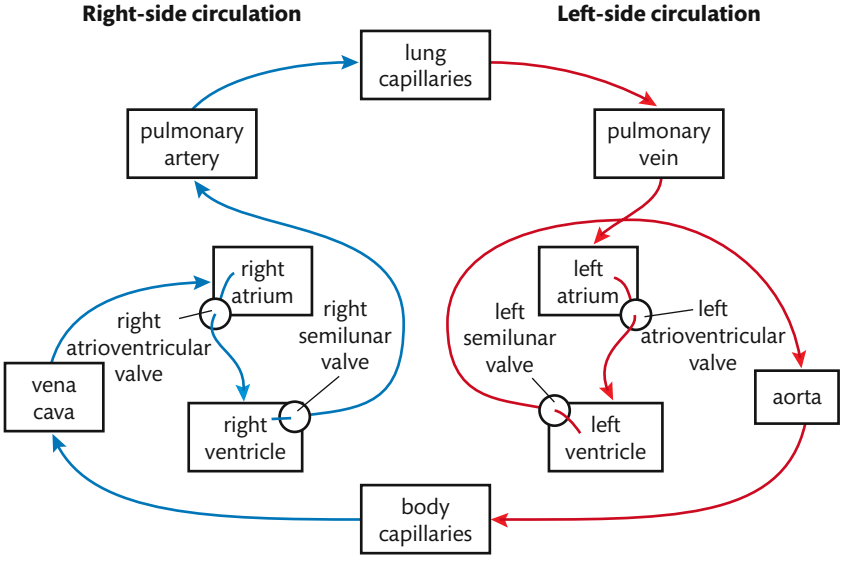


Figure 15.11 A flowchart showing the circulation pattern. Red arrows indicate oxygenated blood and blue arrows indicate deoxygenated blood.

Sometimes a faulty heart valve allows some blood to 'backflow'. The resulting sound when heard through a stethoscope is often described as a 'squishing' sound and is known as a heart murmur.

The sounds of the heart

When you listen directly to the heart using a stethoscope, you can hear a rhythmic set of sounds that most people describe as a series of 'lub dub' sounds. Each 'lub dub' is the sound of one cardiac cycle (one heart beat) and, for the most part, is the sound of the heart valves closing. Remember that the right and left sides of the heart are working in unison, therefore there are only two heart sounds even though there are four heart valves. The atrioventricular valves closing are heard as one sound, 'lub', and the two semilunar valves closing are heard as a second sound, 'dub'. Following these two sounds is a silence before the cycle is repeated.

Artificial heart valves can be surgically implanted to replace damaged natural valves. Artificial and natural valves open and close depending on which side of the valve has the higher blood pressure. The type of replacement valve shown is known as a ball-and-cage design.

Myogenic control of heart rate

If you are at your resting heart rate, your heart itself is controlling the frequency and internal timing of the events of each cardiac cycle. This is called myogenic control. Heart muscle is unusual in that it does not need nervous stimulation to contract. The only control needed from the nervous system is when the heart needs to change its rate of contraction because of increased body activity. The mass of tissue that acts as the living pacemaker for the heart is known as the sinoatrial (SA) node. This node of cells is located in the upper wall of the right atrium, close to where the superior vena cava enters.

The SA node is a group of modified cardiac muscle cells that are capable of generating action potentials at a regular frequency. If your myogenic heart rate is 72 beats min^{-1}, your SA node is generating an action potential every 0.8 seconds. The action potentials from the SA node spread out nearly instantaneously and result in the thin-walled atria undergoing systole. The SA node action potential also reaches a group of cells known as the atrioventricular (AV) node. This node is located in the lower wall of the right atrium, in the septum or partition between the right and left atria.

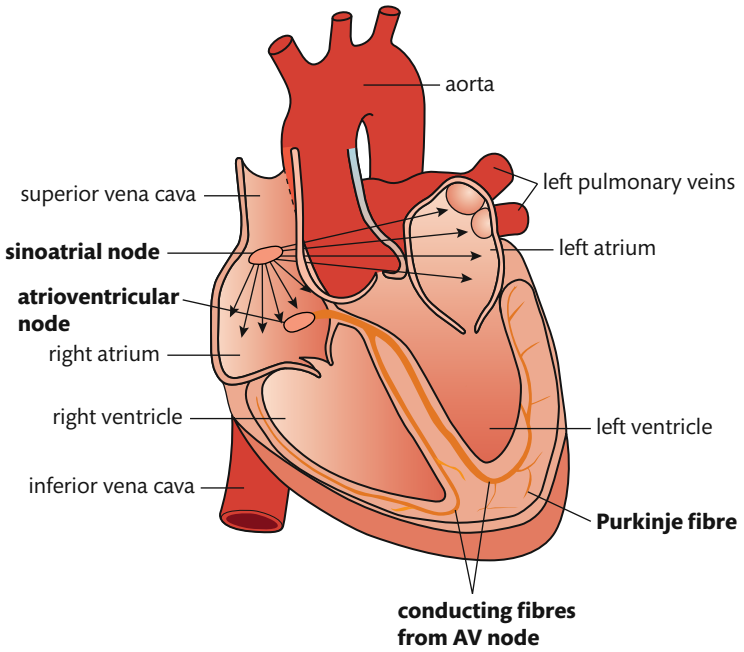


Figure 15.12 This drawing of the human heart shows you the location of the SA node, AV node, and the conducting fibres spreading out through the ventricles from the AV node. The black arrows represent action potentials from the SA node. Cardiac muscle cells are very good at conducting these action potentials through the gap junctions within the intercalated discs that join the cells together. There is a time delay before the AV node sends out action potentials through the conducting fibres that run down the septum between the two ventricles and then to various branches (called Purkinje fibres).

The AV node receives the action potential coming from the SA node and delays for approximately 0.1 second. The AV node then sends out its own action potentials that

spread out to both ventricles. As you learned earlier, the walls of the ventricles are much thicker muscle than the walls of the atria. In order to get the action potentials to reach all of the muscle cells in the ventricles efficiently, there is a system of conducting fibres that begin at the AV node and then travel down the septum between the two ventricles (see Figure 15.12). At various points these conducting fibres have branches called Purkinje fibres that spread out into the thick cardiac muscle tissue of the ventricles. Finally, the gap junctions within the intercalated discs of the cardiac muscle cells finish conducting the impulse and both ventricles undergo systole simultaneously.

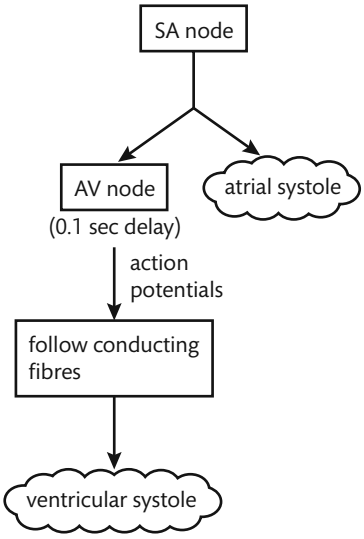


Figure 15.13 A flowchart of the events associated with one heart beat or one cardiac cycle.

When you learn about a mechanism such as the timing of the SA node and the delay before the AV node sends an impulse, think about why the mechanism works in the way that it does. In other words, 'what is the benefit?' In this case, the benefit is to allow the atria time to send the blood down to the ventricles before the AV node 'fires'. This then results in the ventricles contracting and the atrioventricular valves closing, allowing the blood to exit the heart through the semilunar valves.

Mapping the cardiac cycle to a normal ECG trace

An electrocardiogram (ECG) is a graph plotted in real time, with electrical activity (from the SA and AV nodes) plotted on the *y*-axis and time on the *x*-axis. Electrical leads are placed in a variety of places on the skin in order to measure the small voltage given off by these two nodes of the heart. Every repeating pattern on an ECG is a representation of one cardiac cycle. In the previous section you learned that a cardiac cycle is initiated by impulses given off by the SA node. This is where we will begin our 'mapping'.

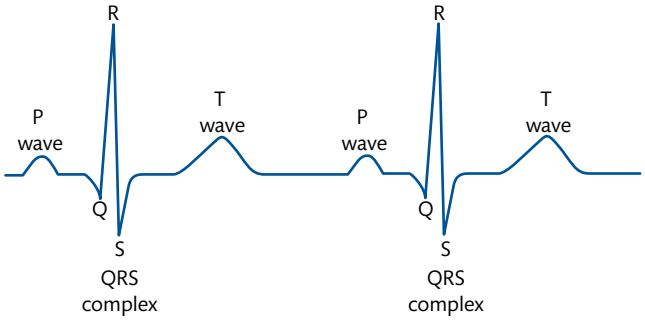


Figure 15.14 An electrical trace of two cardiac cycles (note the repetition from left to right side). Think of this as a graph with electrical activity (measured in millivolts) plotted on the *y*-axis and time plotted on the *x*-axis.

How to 'read' a 'normal' ECG trace (Figure 15.14).

- P wave: this part shows the voltage given off by the SA node, thus it marks atrial systole.
- Point Q: this is the point at which the AV node sends its impulse.
- QRS complex: this is where the impulse from the AV node spreads down the conducting fibres and out to the Purkinje fibres within the ventricles, thus this shows the ventricular systole.
- T wave: the AV node is repolarizing (ions are returning to the resting potential), getting ready to send the next set of impulses for the next cardiac cycle.

Individual cardiac muscle cells grown in a Petri dish contract in an independent rhythm. When heart muscle cells touch each other, they synchronize their contractions. The SA and AV nodes take advantage of this natural ability and provide the timing necessary to synchronize the entire heart.

To learn more about the heart and ECG traces, go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.4.

It is important to be aware of the following.

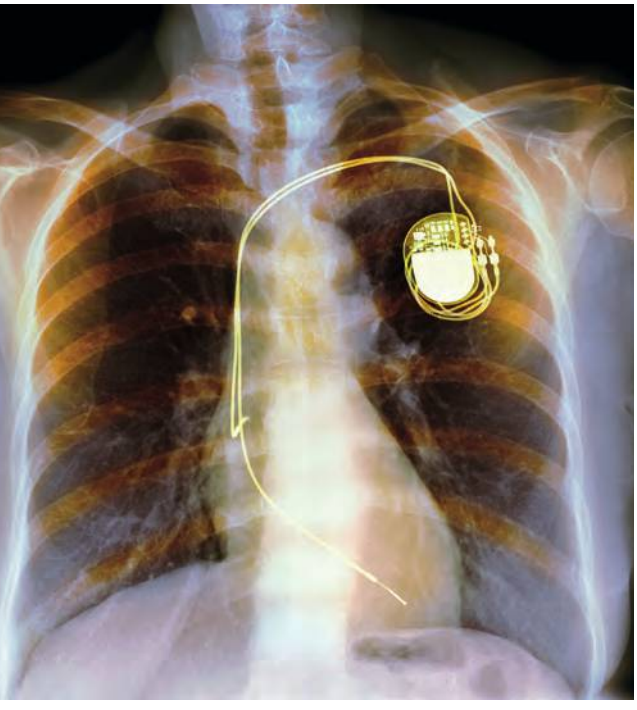
- The SA node also has to repolarize, but the electrical activity is hidden 'behind' the QRS complex.
- An ECG clearly shows the delay between the firing of the SA node and the firing of the AV node. This shows the time separation between the systolic contractions of the atria and ventricles.

Common heart problems and their treatments

The heart is one of the hardest working organs in the body. The only rest that it gets is during the period within the cardiac cycle when any one chamber is not undergoing systole. It is also an organ that cannot stop working for any length of time. Many heart conditions have been studied, and there are now very effective treatments for several of those conditions. We will take a look at some of those conditions and treatments.

Use of artificial pacemakers to regulate heart rate

An artificial pacemaker is a small battery-operated device that is implanted under the skin, typically in the upper chest area. The pacemaker does what the name implies, which is to set the heart rate in the same way that a healthy SA node does naturally. The device is connected to one or more wires (leads) that are threaded into a blood vessel that leads directly into the interior of the heart. The placement of the lead(s) is dependent on the patient's heart problem and how many leads are being placed. The battery-operated device gives off a very small electrical shock at regular intervals, each shock triggering a cardiac cycle. Pacemakers can be used for patients with slow heart rates, fast heart rates, irregular heartbeats, and a host of other problems. The battery life of pacemakers is currently on average 7 years. Patients typically receive an entire new pacemaker when the need arises to ensure that their current pacemaker is still well within the estimated battery life.



A coloured X-ray of the chest of a patient with a dual-lead artificial pacemaker. One lead extends into the right atrium and the other into the right ventricle.

Use of defibrillation devices to treat life-threatening heart conditions

A person suffering from a 'heart attack' may well be suffering from a heart that has stopped (cardiac arrest) or a heart that is no longer in sequence with the set of electrical impulses typical of a cardiac cycle (a condition called arrhythmia). In either case, blood is not being pumped effectively to organs and tissues that are demanding oxygen. Defibrillation is a process carried out using a device that delivers an electric shock to the heart and resets the electrical signals starting with the SA node. When successful, the heart will continue beating on its own once the electrical shock has been delivered.

In recent years, small portable defibrillators have become available and are routinely carried by all medical first responders. These portable defibrillators are called automated external defibrillators (AEDs). It is becoming routine for AEDs to be located in many areas where large numbers of people are routinely found, such as shopping centres, sports stadiums, gymnasiums, etc. AEDs found in these areas are designed for anyone to use because they have audible instructions and the components are very easy to handle.

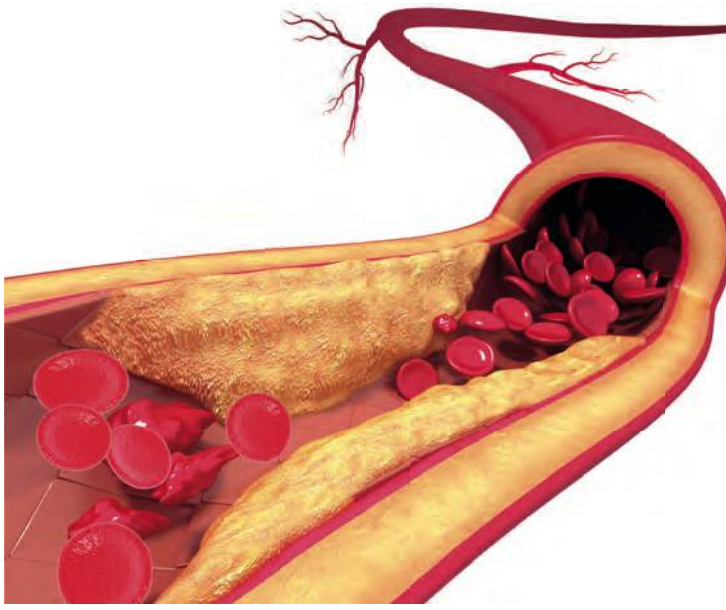
Thrombosis

The term thrombosis refers to the condition when a clot (thrombus) forms within a blood vessel. Some people suffer from a condition called deep vein thrombosis (DVT), where a thrombus develops in one of the larger veins, usually in a leg. Often this occurs when a person has been sitting down for a long period of time, perhaps while travelling on a plane or in a car. The big danger with DVT is that all or a portion of the clot breaks loose and travels to a smaller vein, where a total blockage could occur. This is especially dangerous when the travelling clot lodges in a vein within a lung. DVT is often treated with anticoagulant medications. These are often called blood thinners, but they do not actually 'thin' the blood. Anticoagulants simply help prevent blood clotting from occurring as quickly.

Another form of thrombosis is called coronary thrombosis. Heart muscle needs a rich supply of oxygenated blood to maintain its non-stop action. Heart muscle is supplied with oxygen-rich blood by blood vessels known as coronary arteries. Over time a substance called plaque can build up in one or more these coronary arteries to the point where a substantial narrowing of the lumen (inside) the artery occurs. This can be a problem in itself, but the problems can be increased if a thrombus becomes lodged in the reduced lumen. This can easily lead to a myocardial infarction (heart attack).

TOK

The classic heart symbol has become synonymous with 'love'. There are many ideas about how this association was made. One idea is that, in the 7th century BC, in a Greek and Roman city called Cyrene, the plant known as silphium was used as a form of birth control. The seedpod of the silphium plant has the classic heart shape that we recognize today.



Artwork showing an artery narrowed by plaque build-up over many years. If this blood vessel is feeding oxygenated blood to oxygen-demanding tissue like the cardiac muscle of the heart, a myocardial infarction could result.

Hypertension

Hypertension is higher than 'normal' blood pressure. There is no single blood pressure value that can be used to determine the norm, as a person's blood pressure can be highly variable depending on many factors. Because hypertension typically develops over a period of years, it is best to monitor your blood pressure regularly and look for any increasing trend. Blood pressure measures the force of the blood pushing outwards on the wall of the arteries. The more blood your heart pumps, and the narrower your arteries are, the higher your blood pressure. Loss of elasticity and a build-up of plaque in arteries are prime contributors to hypertension. Even though no single blood pressure reading can be considered to be the norm, the American Heart Association has released ranges of blood pressure values that can be used for advice on cardiovascular health (see Table 15.4). Let's look at what blood pressure is and how it is measured. A blood pressure reading is actually two values, one called the systolic pressure and the other called the diastolic pressure. A typical example might be:

$$\frac{115 \text{ (systolic)}}{68 \text{ (diastolic)}}$$

These values are read as 115 over 68 (both in mm of Hg).

- Systolic pressure: the top number measures the pressure in the arteries when the heart beats (when the heart muscle contracts).
- Diastolic pressure: the bottom number measures the pressure in the arteries when the heart muscle is resting and refilling with blood.

Your blood pressure is typically measured each time you visit the doctor. Many people also monitor their own blood pressure at home with the use of digital sphygmomanometers. After applying the pressure cuff to the upper arm and inflating the cuff, the systolic and diastolic pressures are given by a digital readout.

A digital sphygmomanometer designed for home monitoring of blood pressure.

Table 15.4 The American Heart Association has released the following blood pressure ranges for guidance when interpreting blood pressure readings

Blood pressure category	Systolic mm Hg (upper number)		Diastolic mm Hg (lower number)
Normal	Less than 120	and	Less than 80
Prehypertension	120–139	or	80–89
High blood pressure (hypertension) stage 1	140–159	or	90–99
High blood pressure (hypertension) stage 2	160 or higher	or	100 or higher
Hypertensive crisis (emergency care needed)	Higher than 180	or	Higher than 110

Risk factors affecting coronary heart disease

Coronary heart disease (CHD) is the term used for the slow progression of plaque build-up in arteries and the corresponding problems that can result. Individuals can have CHD for many years without any obvious symptoms, because the early stages do not have noticeable symptoms. Not everyone builds up plaque in their arteries at the same rate. The factors that determine plaque build-up, and thus the eventual chances of heart-related problems, fall into two main categories: those that cannot be controlled or avoided, and those that can.

Most people will have to cope with at least some of the risk factors of CHD during their working life. It is very difficult to measure the effects of any one factor and its impact on the incidence of CHD. Almost all factors have an impact on one or more other factors. For instance:

- people who are overweight often have problems with high blood pressure and cholesterol
- a sedentary lifestyle may lead to obesity
- stress may lead to smoking and overeating, and thus high blood pressure, cholesterol problems, etc.

Researchers who attempt to isolate any one factor and study that factor's impact on CHD must take into account the cascading effect of one factor affecting another, making this type of study open to many interpretations.

Worked example

Epidemiology is defined as the branch of medicine that deals with the incidence, distribution, and possible control of diseases and other factors relating to health. Epidemiological data can be used to help individuals and societies make good choices concerning their own health. Below you will find a brief synopsis of some epidemiological data concerning the incidence of CHD in the UK.

- Coronary heart disease is the most common cause of death in the UK.
- Death rates from CHD have fallen by 45% for people under 65 years of age in the last 10 years.

- The incidence of CHD increases with increasing age.
- The incidence of CHD is higher in men, but is the leading cause of death in women as well.
- Smokers have a 60% higher incidence of mortality as a result of CHD than non-smokers.
- Exposure to passive smoking increases the risk of CHD by 25%.
- Diets high in saturated fat, sodium, and sugar increase the risk of CHD.
- Diets high in complex carbohydrates, fruits, and vegetables decrease the risk of CHD.
- Eating trans-fatty acids (see Section 2.3) increases the risk of CHD.
- Physical activity reduces the risk of CHD.
- High blood pressure may double the risk of mortality from CHD.
- Abnormal blood lipid levels significantly increase the risk of mortality from CHD.
- Obesity significantly increases the risk of mortality from CHD.
- Men with type II diabetes have as much as a four-fold risk of CHD compared with men without type II diabetes; women with type II diabetes have as much as a five-fold risk of CHD compared with women without type II diabetes.
- Ethnicity has a significant impact on CHD risk. People from India, Pakistan, Bangladesh, and Sri Lanka living in the UK have a 50% higher incidence of mortality from CHD compared with other ethnic groups.
- First-generation relatives of patients who have suffered a heart attack have double the risk of CHD compared with those whose parents did not suffer a heart attack.

After reading through this information, create a list of bullet points that offer advice to people to help them make good lifestyle choices in order to reduce their chances of developing CHD. Notice that some of the information given cannot be acted upon by an individual (e.g. age/gender/ethnic background/family history) and so this information cannot be used to help someone follow a healthy lifestyle, although it can make them aware of the importance of those factors that can be controlled.

Solution

In order to lead a healthy lifestyle, specifically designed to minimize the risk of CHD:

- do not smoke or be in an area where cigarette smoke is present
- eat a healthy diet minimizing saturated fats, trans-fats, salt, and sugar, while increasing your intake of fruits, vegetables, and complex carbohydrates
- attempt a reasonable amount of physical activity as often as possible
- attempt to lower high blood pressure by natural means or, if necessary, by taking prescription medicines
- keep cholesterol and other blood lipids in a normal range by eating a healthy diet and/or taking prescription medications
- make lifestyle choices that will lead to weight loss, if necessary
- avoid lifestyle choices that could lead to type II diabetes, or control the disease as much as possible.

Exercises

13 Artificial hearts and heart valves have been designed and surgically implanted into both test animals and humans. How do the valves within these artificial devices 'know' when it is time to close and open?

14 An ECG is a graph showing the electrical activity of the heart. The voltage can be traced back to the SA node and the AV node. When a person exercises and thus increases his or her heart rate, what is the expected change in a subsequent ECG?

15 Why is there a delay between the signal from the SA node and the signal from the AV node within one cardiac cycle?

16 Why are heart cells so efficient at passing an electrical signal from cell to cell?

NATURE OF SCIENCE

Cooperation and collaboration between groups of scientists: the International Council for the Control of Iodine Deficiency Disorders includes a number of scientists who work to eliminate the harm done by iodine deficiency.

D.5 Hormones and metabolism

Understandings:

- Endocrine glands secrete hormones directly into the bloodstream.
- Steroid hormones bind to receptor proteins in the cytoplasm of the target cell to form a receptor–hormone complex.
- The receptor–hormone complex promotes the transcription of specific genes.
- Peptide hormones bind to receptors in the plasma membrane of the target cell.
- Binding of hormones to membrane receptors activates a cascade mediated by a second messenger inside the cell.
- The hypothalamus controls hormone secretion by the anterior and posterior lobes of the pituitary gland.
- Hormones secreted by the pituitary control growth, developmental changes, reproduction, and homeostasis.

Applications and skills:

- Application: Some athletes take growth hormones to build muscles.
- Application: Control of milk secretion by oxytocin and prolactin.

Overview of the endocrine system

Endocrine glands produce and secrete hormones. Hormones are chemical messengers that usually have a physiological effect far from their gland of origin and thus are transported throughout the body by the bloodstream. The cells that are affected by any one hormone are referred to as target cells of that hormone. Some endocrine glands occur in pairs, such as the adrenal glands (see Figure 15.15), and some are singular glands, such as the pancreas. The pancreas is the only gland that has both exocrine and endocrine functions.

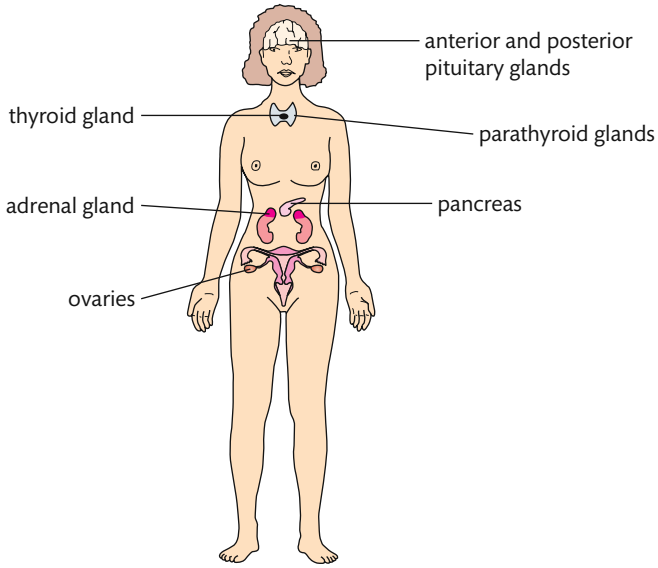


Figure 15.15 Some endocrine glands are shown in this body outline. All endocrine glands produce one or more hormones.

NATURE OF SCIENCE

Hormones produced by your thyroid need the element iodine for their structure. Without iodine these hormones cannot be synthesized. Approximately one-third of the world's population resides in areas where there is a deficiency of iodine. Without supplemental iodine, people in these areas can suffer several conditions, including severe brain damage. One solution is to provide iodized salt to these people. This sounds simple but a problem is the huge number of people involved. An organization called The International Council for the Control of Iodine Deficiency Disorders is attempting to solve iodine deficiency worldwide.

Each hormone produced by an endocrine gland has one or more tissue type in the body that is the 'target tissue' of that hormone. In many instances the target tissue is located far away from the endocrine gland. Thus endocrine glands secrete hormones into the blood for dispersal to all cells of the body, even though only the target tissue cells are affected by the hormone.

Steroid hormones

Steroid hormones are typically synthesized from cholesterol and are classified as lipids. Therefore steroids have the chemical and solubility properties of a lipid. You will recall that a plasma membrane (or any cell membrane) is a double layer of phospholipids (see Section 1.3). This means that steroids easily pass through cell membranes because both steroids and phospholipid molecules are relatively non-polar. Once a steroid hormone has entered the cytoplasm of a cell, it binds with a receptor protein and forms what is called a receptor–hormone complex. In the simplest scenario, this receptor–hormone complex then passes through the nuclear membrane and selectively binds to one or more specific gene. In some instances, the complex inhibits transcription, and in other cases the complex promotes transcription. In this way, steroid hormones control the production of proteins within the target cell. The target cells of steroid hormones have their biochemistry dramatically altered as a result of the presence of the hormone. Examples of naturally occurring steroid hormones include oestrogen, progesterone, and testosterone.

A man suffering one of the consequences of a lack of iodine in the diet. The large growth on his neck is called a goitre. It is the result of the growth of the thyroid gland in an attempt to compensate for not being able to produce enough thyroxine because of a deficiency of iodine in the diet.

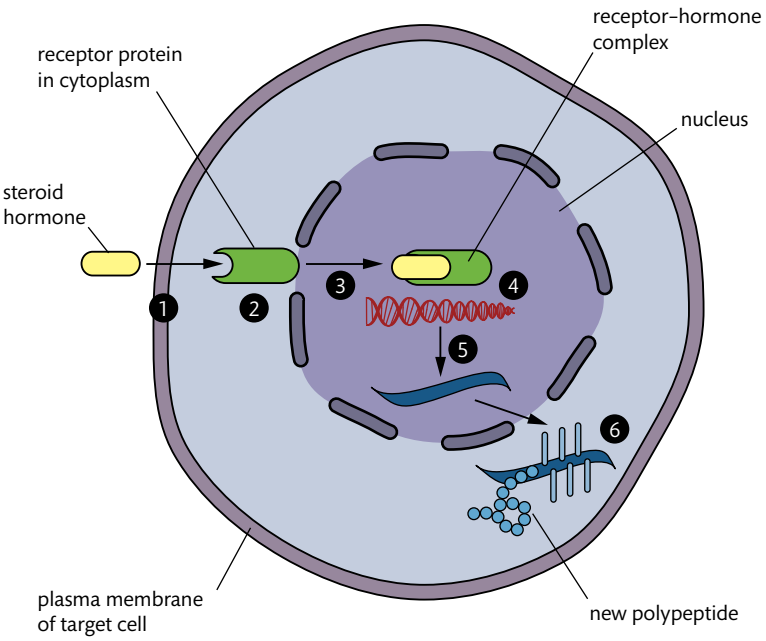
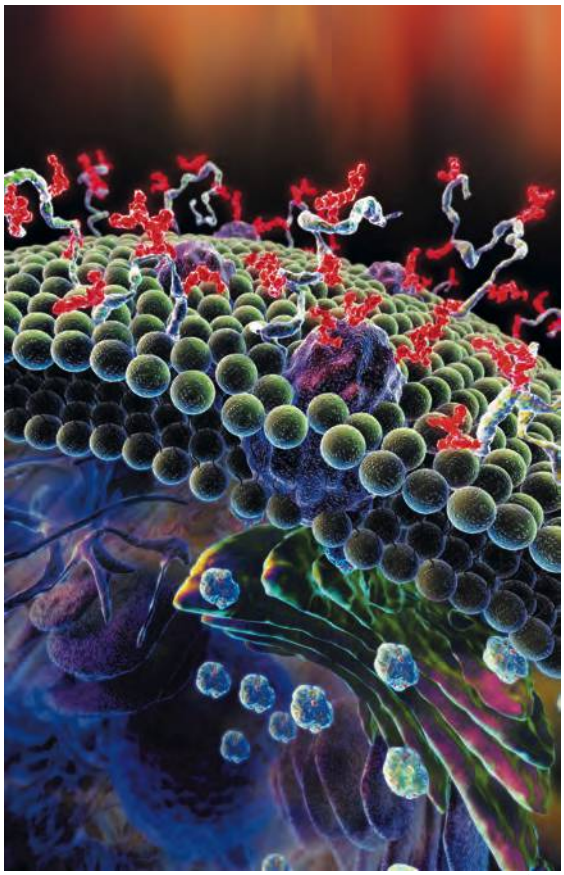


Figure 15.16 An illustrated version of the general mechanism of a steroid hormone. (1) A non-polar (lipid soluble) steroid hormone enters directly through biphospholipid layer of plasma membrane. (2) A steroid hormone binds to a receptor protein in the cytoplasm to make a receptor–hormone complex. (3) The receptor–hormone complex enters the nucleus through a nuclear pore. (4) The receptor–hormone complex binds to a specific gene of DNA and, in this example, promotes transcription for this gene. (5) Messenger (m)RNA molecules are synthesized as a result. (6) Ribosomes on the endoplasmic reticulum translate mRNA into a new polypeptide.



Artwork showing a plasma membrane with a variety of proteins on its surface. Some of these proteins may be receptors for peptide hormones.

Peptide hormones

Peptide hormones get their name from the fact that they are composed of amino acids and thus are proteins. When a peptide hormone reaches a target cell, the hormone binds to a receptor protein on the outer surface of the cell membrane. The presence or absence of the hormone's receptor protein determines whether or not a cell is a target cell of that particular hormone. Similar to an enzyme and substrate, there must be a molecular shape and charge 'fit' between the peptide hormone and its receptor molecule. Once a peptide hormone has chemically bonded to a receptor protein, a secondary messenger molecule is triggered into action in the cytoplasm of the cell. Often the secondary messenger then chemically activates one or more other messenger molecules in the cytoplasm in a cascade of reactions.

The final messenger molecule at the end of the cascade of reactions will typically accomplish one of two possibilities:

1. the final messenger activates an enzyme in the cytoplasm, and thus a reaction proceeds that was not possible before the peptide hormone began this sequence, or
2. the final messenger molecule activates a transcription factor that enters the nucleus and either promotes or inhibits the transcription phase of protein synthesis.

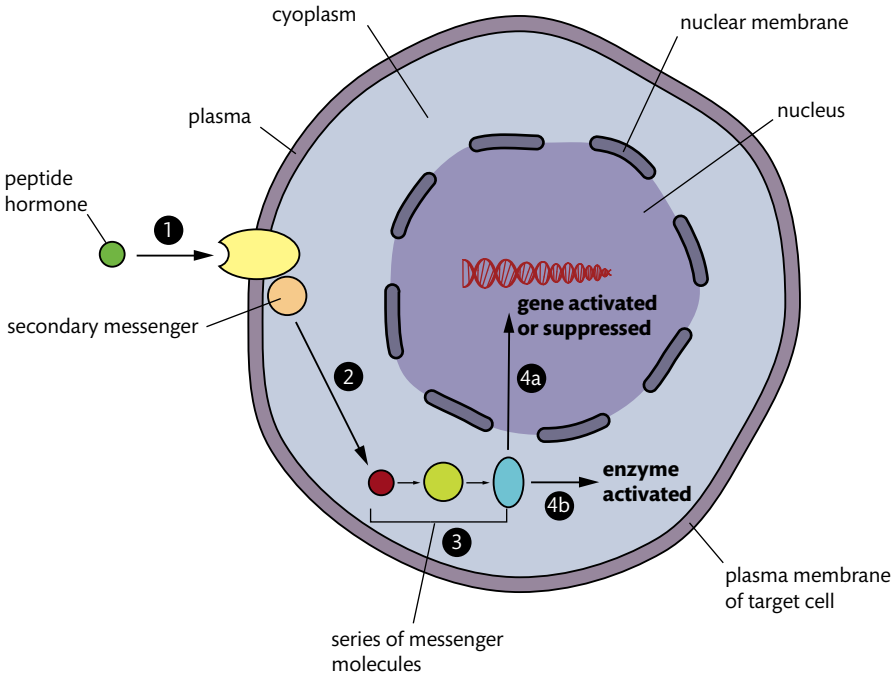


Figure 15.17 An illustrated version of the general mechanism of a peptide hormone. (1) A peptide hormone fits the complementary shape and charge of a receptor protein within the plasma membrane of a target cell. (2) A receptor protein signals the beginning of a cascade of reactions. (3) A series of second messenger molecules is activated. (4a) One possible consequence is a second messenger molecule that promotes or inhibits a gene, leading to more or less of a polypeptide being synthesized. (4b) A second possibility is that an enzyme is activated and a reaction or reaction sequence begins that is catalysed by that activated enzyme.

One of the fundamental differences between steroid and peptide hormones is whether the hormone actually enters the target cell that it acts upon. Steroid hormones do enter the target cell and bind to a receptor protein in the cytoplasm, whereas peptide hormones interact with a receptor protein on the outside of the plasma membrane of a target cell.

The pituitary gland and its 'boss', the hypothalamus

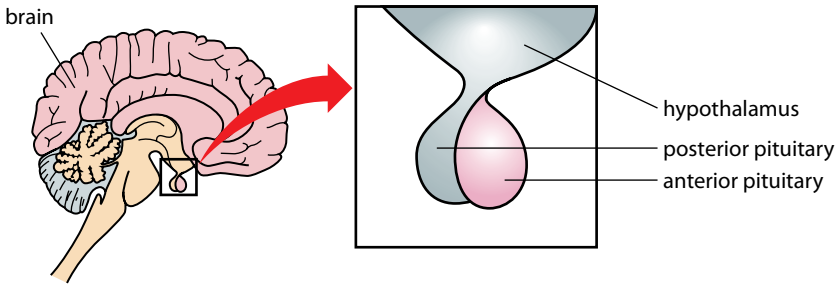


Figure 15.18 The position of the hypothalamus and pituitary.

It is common to read that the pituitary gland is the 'master gland'. It is true that the pituitary gland produces many different hormones, and some of those hormones influence the production and secretion of other hormones, but the pituitary itself is largely controlled by the action of the nearby hypothalamus (see Figure 15.18). Most people refer to the pituitary gland as a singular gland, but it is actually two glands that exist as different 'lobes'. The anterior and posterior lobes of the pituitary communicate with the hypothalamus in different ways.

The posterior lobe of the pituitary contains the axons of cells called neurosecretory cells (see Figure 15.19). These are very long cells whose dendrites and cell bodies are located in the hypothalamus and whose axons extend down into the posterior pituitary. Hormones, such as oxytocin and ADH, are produced at the cell body end of these cells (in the hypothalamus) and then move down the axons into the posterior pituitary gland. They are then secreted in a similar way to the release of a neurotransmitter. This explains why these hormones are said to have been produced within the hypothalamus, but they are in fact secreted from the posterior pituitary.

In a test question referring to oxytocin and/or antidiuretic hormone (ADH), specifically look for the words 'produced' versus 'secreted' before answering a question concerning the origin of these two hormones.

The relationship between the hypothalamus and the anterior pituitary works differently (see Figure 15.19). The hypothalamus contains capillary beds that take in hormones produced by the hypothalamus itself. These hormones are also produced by neurosecretory cells, but these cells are located entirely within the hypothalamus. These hormones are often referred to as releasing hormones, for example gonadotropin-releasing hormone (GnRH). The capillary beds join together into a blood vessel known as a portal vein. This vein extends down into the anterior pituitary. Here, the portal vein branches into a second capillary bed that allows the releasing hormones to leave the bloodstream for their target cells, the cells of the anterior pituitary. The releasing hormones stimulate the anterior pituitary cells to secrete specific hormones. For example, GnRH stimulates the secretion of both follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The hormones produced by the anterior pituitary enter the bloodstream through the same capillary beds that allowed the releasing hormones to exit. As you learned for the reproductive system, the target cells of LH and FSH are the gonads of both females and males (see Section 6.6).

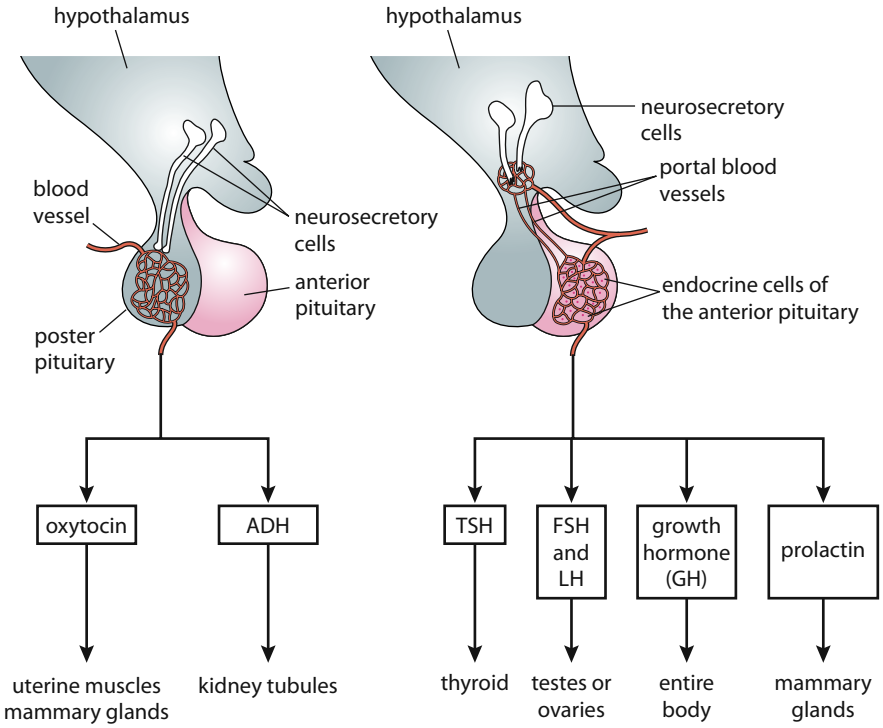


Figure 15.19 The posterior pituitary (left) with its hormones and relationship with the hypothalamus via neurosecretory cells. The anterior pituitary (right) with its connection to the hypothalamus via portal blood vessels.

The portal veins that connect the capillary beds within the hypothalamus to capillary beds in the anterior pituitary comprise one of three places in the body where a portal system of circulation is used (two capillary beds in one circuit). The other two are the portal circulation connecting the villi capillary beds to the sinusoids of the liver, and the glomerulus capillary bed connecting the peritubular capillary bed of a nephron.

TOK What do you know about some of the foods that you eat? Are you aware that some of the foods you eat may very well contain genetically modified crops? Do you know whether the meat you are eating was taken from an animal that was fed antibiotics or steroids and/or growth hormones? Is this food safe? Who determines whether it is safe?

To learn more about genetically modified crops, go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.5.

Control of milk secretion by prolactin and oxytocin

During a mammal's pregnancy there are many hormone changes that help control the various processes necessary for foetal development, birth, and postpartum (post-birth). Some of the hormonal changes are necessary for lactation. The two hormones that are most directly involved in lactation are two pituitary hormones, prolactin and

oxytocin. During pregnancy, increasing levels of prolactin result in the development of the milk-producing cells within the breast. The naturally high levels of oestrogen during pregnancy inhibit those cells from releasing milk. After birth, two events stimulate the secretion of milk so that breastfeeding can begin. One is the drastic lowering of oestrogen as a result of the birthing process, and the second is the high levels of oxytocin that stimulated the uterine contractions. Without the inhibiting effects of oestrogen, prolactin stimulates the milk-producing cells of the breasts to begin releasing the milk. In addition, oxytocin results in the contraction of smooth muscle tissue surrounding the ducts carrying the milk, which results in milk ejection. The production of both hormones is increased by the stimulation of the breast nipple caused by a suckling infant. This is a form of physiological control known as positive feedback. This also explains why a woman who does not breastfeed her child soon does not produce breast milk.

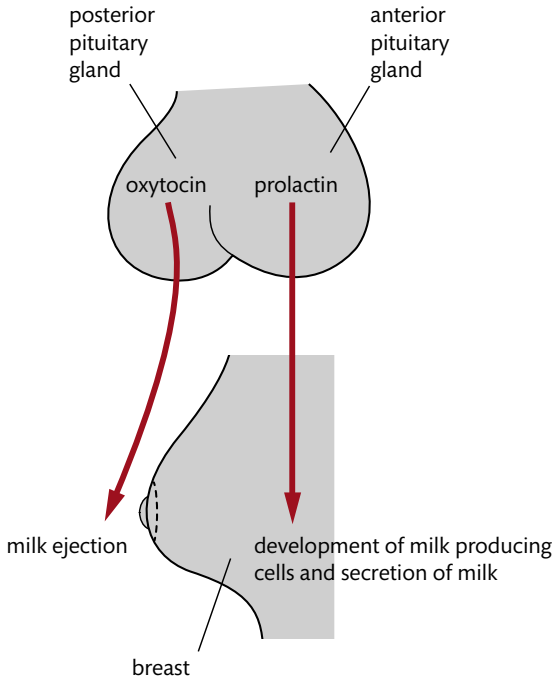


Figure 15.20 A simplified diagram showing the effects of prolactin and oxytocin on milk production, secretion, and ejection.

The first 'milk' produced and secreted after birth is called colostrum. Colostrum is high in carbohydrates, proteins, and antibodies, but low in fat, because newborns are not efficient at digesting fats. Colostrum levels decrease over the first few days of breastfeeding as the more typical breast milk begins to be produced.

CHALLENGE YOURSELF

2 For each of the following hormones, state where the hormone is produced, where it is secreted, and the target tissue of the hormone.
- **(a)** Antidiuretic hormone (ADH).
- **(b)** Follicle-stimulating hormone (FSH).
- **(c)** Progesterone.
- **(d)** Oxytocin.

Other pituitary gland hormones and their functions

Nine different peptide hormones are secreted from the pituitary gland. We have considered a few of these hormones and their effects within the body. Most of these hormones have been described in earlier sections. Table 15.5 will remind you of their functions.

Table 15.5 Selected hormones produced by the two lobes of the pituitary gland

Controls	Hormone(s)	Functions in brief
Reproduction	LH (luteinizing hormone) and FSH (follicle-stimulating hormone)	Prepares ovarian cells for ovulation in females, and needed for sperm production in males
Growth	GH (growth hormone)	Stimulates mitosis and organism growth
Developmental changes	GH, LH, FSH	GH is necessary for all developmental growth throughout adulthood. LH and FSH secretions increase during puberty, leading to ovulation and sperm production, among other functions
Homeostasis	ADH (antidiuretic hormone)	Secretion of ADH is needed for the reabsorption of water from the collecting ducts in the kidneys, it is therefore involved in the homeostatic mechanisms of osmoregulation

Unfortunately, many athletes in many sports have given in to the temptation of using performance-enhancing drugs (PEDs). We frequently hear about this abuse during some of the larger international competitions, such as the Olympics and the Tour de France. Many PEDs are hormones, including a variety of steroids and also GH.

Exercises

17 Differentiate between the actions of peptide hormones and steroid hormones.

18 In an earlier section, you learned about exocrine glands. In this section, you learned about endocrine glands. Differentiate between these two types of glands.

19 Why do you need to be careful about using the phrases 'produced by' and 'secreted by' when referring to the hormones associated with the posterior pituitary?

20 Prolactin is a hormone that is produced throughout most of a woman's pregnancy. This hormone results in milk production and secretion. Why doesn't a pregnant woman secrete milk before giving birth to her child?

NATURE OF SCIENCE

Scientists have a role in informing the public: scientific research has led to a change in public perception of smoking.

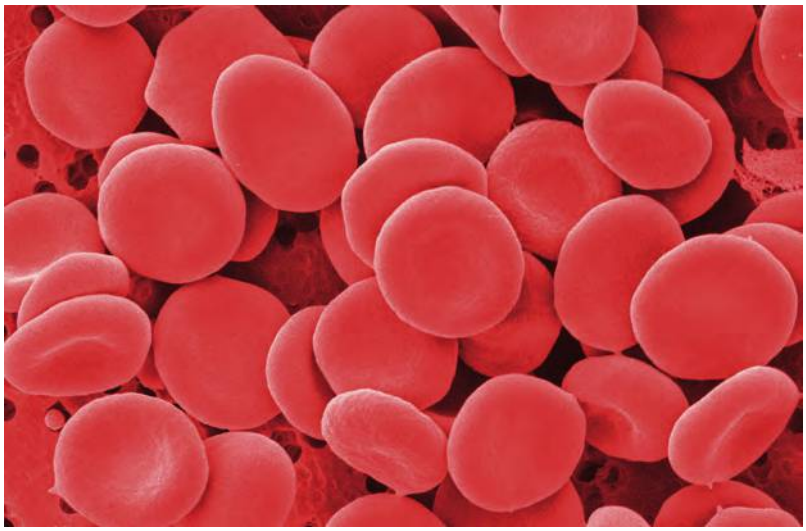
D.6 Transport of respiratory gases

Understandings:

- Oxygen dissociation curves show the affinity of haemoglobin for oxygen.
- Carbon dioxide is carried in solution and bound to haemoglobin in the blood.
- Carbon dioxide is transformed in red blood cells into hydrogen carbonate ions.
- The Bohr shift explains the increased release of oxygen by haemoglobin in respiring tissues.
- Chemoreceptors are sensitive to changes in blood pH.
- The rate of ventilation is controlled by the respiratory control centre in the medulla oblongata.
- During exercise the rate of ventilation changes in response to the amount of carbon dioxide in the blood.
- Foetal haemoglobin is different from adult haemoglobin, allowing the transfer of oxygen in the placenta onto the foetal haemoglobin.

Applications and skills:

- Application: Consequences of high altitude for gas exchange.
- Application: pH of blood is regulated to stay within the narrow range of 7.35 to 7.45.
- Application: Causes and treatments of emphysema.
- Skill: Analysis of dissociation curves for haemoglobin and myoglobin.
- Skill: Identification of pneumocytes, capillary endothelium cells, and blood cells in light micrographs and electron micrographs of lung tissue.



Erythrocytes have no nucleus and few organelles. Each erythrocyte contains around 250 million haemoglobin molecules.

Haemoglobin

Haemoglobin is the protein molecule found within erythrocytes that is responsible for carrying most of the oxygen within the bloodstream. Each erythrocyte is basically a plasma membrane surrounding cytoplasm filled with haemoglobin molecules. The erythrocytes have no nuclei and few organelles or other components other than haemoglobin. Each haemoglobin molecule is capable of reversibly binding to as many as four oxygen molecules and one carbon dioxide molecule.

Each haemoglobin molecule is composed of four polypeptides. Each polypeptide has a haem group near its centre, and each haem group has an iron atom within it (see Figure 15.21). When haemoglobin reversibly binds to an oxygen molecule, it is the iron atom within the haem group that is bonding with the oxygen. Because haemoglobin has a total of four iron atoms within four haem groups within four polypeptides, it has the capability of transporting a maximum of four oxygen molecules ($4O_2$).

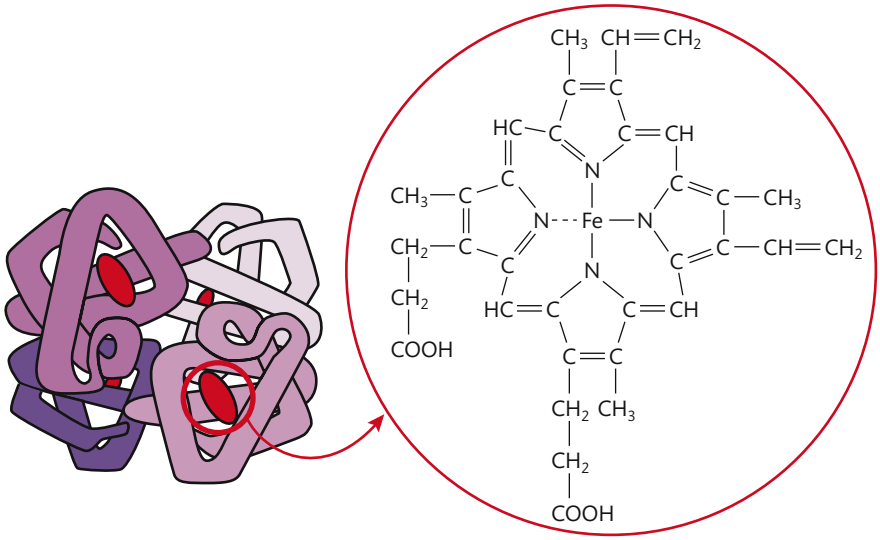


Figure 15.21 Haemoglobin is a large protein consisting of four polypeptides with a haem group within each. The molecular structure of a haem group is shown on the right. Notice the iron atom at the centre of the molecule.

Carbon monoxide is a by-product of the combustion of many fuels. Haemoglobin has a greater affinity for carbon monoxide than for oxygen. People breathing carbon monoxide are depriving their tissues of oxygen as the carbon monoxide molecules bind to haemoglobin and prevent haemoglobin from carrying a normal load of oxygen. Carbon monoxide poisoning can be fatal. Some homes and businesses are equipped with carbon monoxide monitoring devices to protect occupants from this silent and odourless killer.

Haemoglobin changes shape and affinity when carrying oxygen

You will recall that proteins have an ability to change their three-dimensional shape under certain circumstances. For example, the induced-fit hypothesis of enzyme catalysis proposes that an enzyme changes shape as the substrate enters the enzyme's active site. A similar phenomenon occurs when oxygen binds to haemoglobin. Haemoglobin actually has four possible shapes, depending on how many oxygen molecules are bound to the iron atoms of the haem groups. These different shapes affect the haemoglobin's ability to bind with oxygen molecules. This is known as haemoglobin's affinity for oxygen. The greater the tendency to bind with oxygen, the higher the affinity.

Haemoglobin molecules that are already carrying three oxygen molecules have the greatest affinity for oxygen. Conversely, haemoglobin molecules that are carrying no oxygen molecules have the least affinity for oxygen. You might think that this does not make sense, but it does when you learn that each oxygen molecule that binds to haemoglobin changes the haemoglobin's shape in a way that increases its affinity for another oxygen molecule. Haemoglobin can carry a maximum of four oxygen molecules, so one that is already carrying four oxygens has no affinity for oxygen.

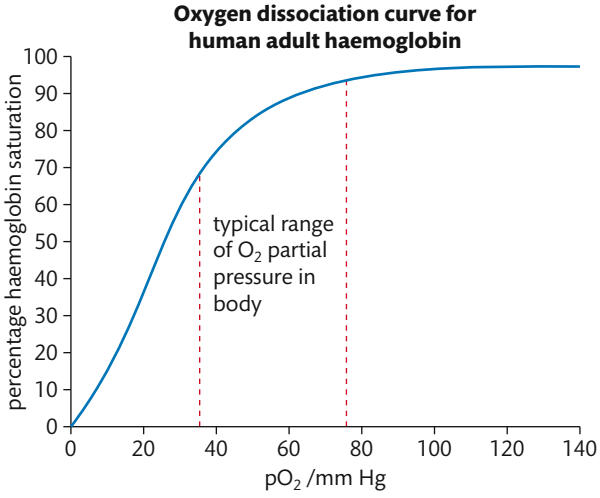
A common abbreviation that is used for haemoglobin is Hb_4. Each molecule of oxygen that is bound to Hb_4 adds two oxygen atoms. Thus, haemoglobin's affinity for oxygen from lowest to highest is: Hb_4, Hb_4O_2, Hb_4O_4 and, finally, Hb_4O_6.

To learn more about haemoglobin and oxygen dissociation curves, go to the hotlinks site, search for the title or ISBN, and click on Chapter 15: Section D.6.

Oxygen dissociation curves

Oxygen dissociation curves are graphs that show how various forms of haemoglobin or myoglobin perform under various conditions. The *x*-axis of these graphs measures the partial pressure of oxygen. Partial pressure is the pressure exerted from a single type of gas when it is found within a mixture of gases. The air that we breathe is a mixture of gases, and oxygen is just one component of this mixture. Within our bloodstream and in our body tissues is a different mixture of gases, and once again oxygen is just one component of that mixture. The mixture of gases exerts an overall (total) pressure; the portion of the total pressure that is caused by oxygen alone is the partial pressure of oxygen.

Figure 15.22 This oxygen dissociation curve shows the range of oxygen partial pressure found in the body. Partial pressures are often given in kPa (kilopascals) rather than mm Hg (millimetres of mercury).



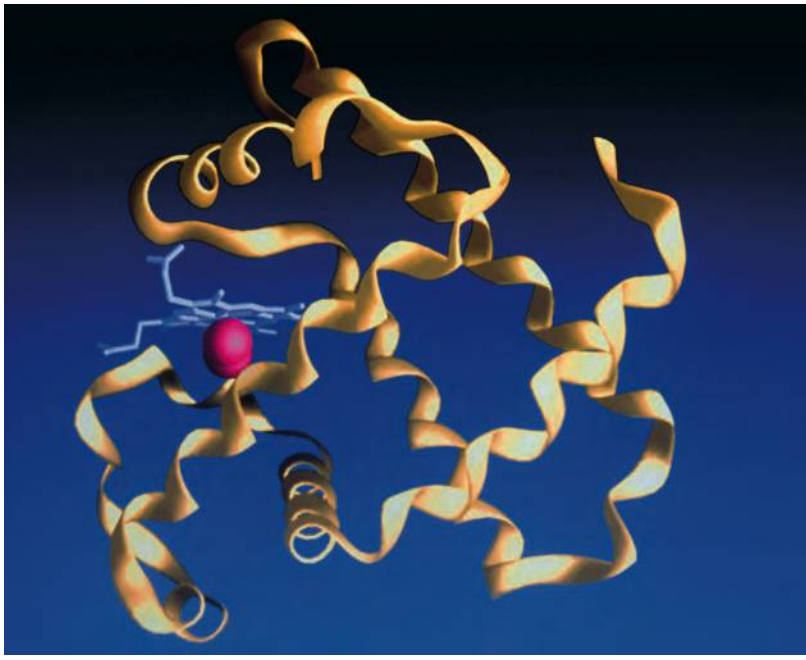
The *y*-axis of an oxygen dissociation curve shows the percentage saturation of haemoglobin with oxygen. Haemoglobin is not saturated until it is carrying (bonded to) four oxygen molecules. Let's look at the oxygen dissociation curve for human adult haemoglobin (Figure 15.22).

Notice the very steep S-shape of the graph. This shape is indicative of the affinity changes for oxygen that haemoglobin undergoes when at least some oxygen is already bound to the molecule. At the lower end of the graph, little oxygen is already bound and this gives the shape of the lower portion of the 'S'. In the upper half of the graph, haemoglobin is already bound to some oxygen and has increased its affinity for oxygen (because of the

protein shape change), and the graph is very steep in that area until nearly all of the haemoglobin is saturated.

Notice on the graph the homeostatic range of oxygen partial pressures within the body. The upper end of the normal range (about 75 mm Hg or 10 kPa) is the oxygen partial pressure found within the lungs. The graph shows that more than 90% of the haemoglobin becomes saturated with oxygen within the lungs. At the lower end of the normal range (about 35 mm Hg or 5 kPa), only about 50% of the haemoglobin is still saturated with oxygen. This partial pressure of oxygen is more typical of body tissues that have actively undergone cell respiration. This means that 40–50% of the haemoglobin that has recently been to the lungs gives up (dissociates) one or more oxygen molecules when the haemoglobin reaches the body tissues. Haemoglobin molecules typically do not 'empty' their oxygen load when they reach respiring body tissues, but they do release a significant amount of oxygen within a relatively narrow range of oxygen partial pressures. It is this release (dissociation) of oxygen that gives these graphs their name: oxygen dissociation curves.

Oxygen dissociation curves show the tendency of haemoglobin to bind to oxygen (affinity) and separate from oxygen (dissociate).



Myoglobin molecular structure. The ribbon-like structure in this model is a single polypeptide chain. Centre left is the haem group (blue) with bound oxygen (red).

Comparison of haemoglobin and myoglobin

Myoglobin is an oxygen-binding protein found in muscles. Each myoglobin molecule consists of a single polypeptide, a haem group, and an iron atom. Each myoglobin can bind to only one oxygen molecule. The function of myoglobin is to store oxygen within muscle tissues until muscles begin to enter an anaerobic situation when exercising heavily. Then, and only then, does myoglobin dissociate its oxygen and thus delay the onset of lactic acid fermentation.

Look at Figure 15.23. Notice that myoglobin's position on this graph is to the left of haemoglobin. Except for the very upper end of the oxygen partial pressure scale, any point selected on the *x*-axis will show myoglobin still bound to its oxygen when haemoglobin has dissociated oxygen. This ability of myoglobin to 'hold onto' its oxygen, even at low oxygen partial pressures, allows myoglobin to serve its function of delaying tissues going into anaerobic conditions. You can think of myoglobin as providing a final reservoir of oxygen when you are exercising heavily.

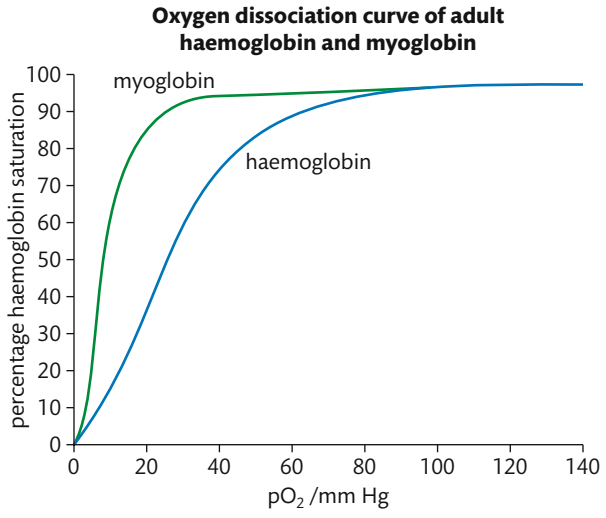


Figure 15.23 Oxygen dissociation curves of haemoglobin and myoglobin. Myoglobin dissociates oxygen only when the oxygen partial pressure gets very low, e.g. in actively respiring muscle tissues.

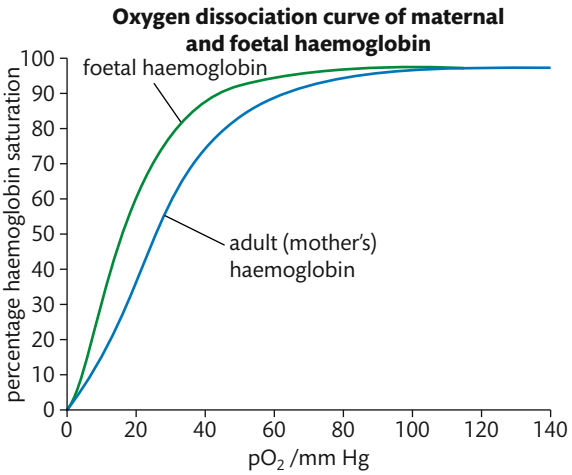


Figure 15.24 Foetal haemoglobin has a greater affinity for oxygen than adult haemoglobin in the range of partial pressures typical of human tissues.

Comparison of adult haemoglobin and foetal haemoglobin

The haemoglobin produced by a foetus is slightly different in molecular composition compared with adult haemoglobin. This is because the haemoglobin of a foetus must have a greater affinity for oxygen than adult haemoglobin. This is so that, in the placental capillaries, adult haemoglobin is more likely to dissociate oxygen, and foetal haemoglobin is more likely to bind to that same oxygen. Foetal haemoglobin dissociates this oxygen only when it reaches the respiring tissues of the foetus.

In Figure 15.24, notice that the curve for foetal haemoglobin is consistently to the left of adult haemoglobin. Any point selected on the *x*-axis shows that adult haemoglobin binds less oxygen at that partial pressure compared with foetal haemoglobin.

The Bohr shift

Haemoglobin's affinity for oxygen is reduced in an environment where carbon dioxide partial pressure is high. Such an environment is found in body tissues that are actively undergoing cell respiration. Another way of saying this is that the haemoglobin is induced to release (dissociate) oxygen within the capillaries of body tissues. This effect is called the Bohr shift and results when carbon dioxide binds to haemoglobin, causing a shape change that promotes the release of oxygen.

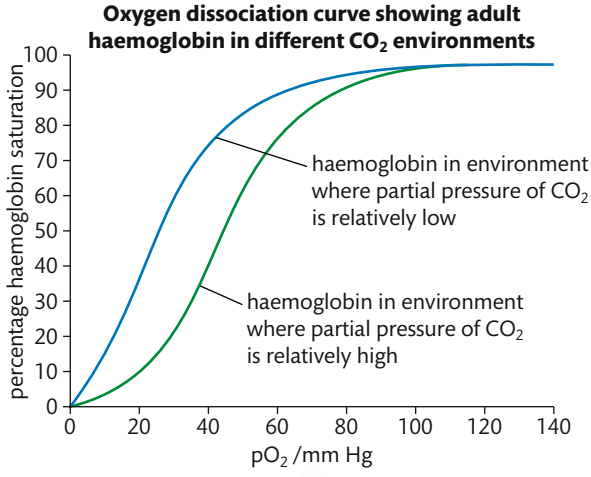


Figure 15.25 The Bohr shift. Haemoglobin is more likely to dissociate oxygen in an environment where carbon dioxide partial pressure is high.

Let's consider what happens to adult haemoglobin in different environments in the body. Look at Figure 15.25. The curve on the left shows what happens to haemoglobin passing through the lungs, an environment where the partial pressure of carbon dioxide is relatively low. In such an environment, oxygen binds easily to haemoglobin. The curve on the right shows what happens to haemoglobin in actively respiring tissues that are giving off carbon dioxide as a waste product. The carbon dioxide is entering the bloodstream and some is binding with haemoglobin. In this situation, oxygen is more likely to dissociate from haemoglobin at any oxygen partial pressure. This is the Bohr shift. It promotes the release of oxygen within body tissues and the binding of oxygen within the lungs, both situations using the same molecule.

The Bohr shift is a good example of an evolutionary adaptation that benefits organisms at a molecular level.

Carbon dioxide transport in the blood

Cell respiration is a process that links all living organisms: sugars, such as glucose, are oxidized in order to generate ATP molecules. The primary waste product of this process is carbon dioxide. In humans, as well as many other organisms, this carbon

dioxide diffuses out of a respiring cell and eventually enters a nearby capillary bed. Once carbon dioxide enters the bloodstream, there are three ways in which it is transported to the lungs:

- a small percentage of carbon dioxide remains as it is and simply dissolves in the blood plasma
- some carbon dioxide enters erythrocytes and becomes reversibly bound within haemoglobin (each haemoglobin can carry a single carbon dioxide molecule; this is the basis of the Bohr shift)
- most (approximately 70%) of the carbon dioxide enters erythrocytes and is converted into hydrogen carbonate ions, which then move into the blood plasma for transport.

Formation of hydrogen carbonate ions

The cytoplasm of erythrocytes contains an enzyme known as carbonic anhydrase. This enzyme catalyses a reaction in which carbon dioxide and water combine to form carbonic acid (H_2CO_3). Carbonic acid then dissociates into a hydrogen carbonate ion and a hydrogen ion (see Figure 15.26).

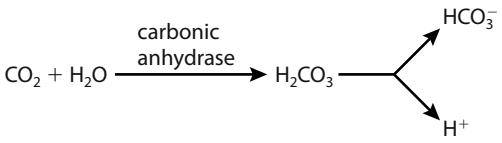


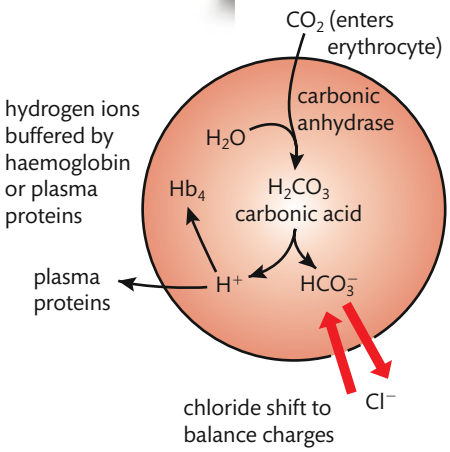
Figure 15.26 Carbonic anhydrase catalyses the formation of carbonic acid and therefore the spontaneous formation of hydrogen carbonate.

The hydrogen carbonate ions formed from this reaction exit the cytoplasm of the erythrocyte through specialized protein channels in the erythrocyte membrane. The transport mechanism is facilitated diffusion, and it works by a mechanism that exchanges one hydrogen carbonate ion moving out of the erythrocyte into the blood plasma, for one chloride ion moving into the erythrocyte from the blood plasma. This exchange of the two negative ions keeps a balance of charges on either side of the erythrocyte plasma membrane, and is known as the chloride shift (see Figure 15.27).

Maintaining a narrow homeostatic range of pH in the blood

The pH of blood plasma must be regulated in order to maintain a narrow range of 7.35 to 7.45. This requires buffering mechanisms, because many more hydrogen ions are produced when an individual is exercising, as a result of the increased production of carbon dioxide. The hydrogen ions that are produced because of the dissociation of carbonic acid must not be allowed to stay in solution in either the erythrocyte cytoplasm or in the blood plasma. The temporary removal of hydrogen ions from these solutions is called pH buffering. Notice that when carbonic acid dissociates within the cytoplasm of the erythrocyte, some of the resulting hydrogen ions can become temporarily bound at various places on haemoglobin molecules, and thus are taken 'out of solution'. Many of the hydrogen ions that exit the erythrocyte bind with proteins circulating as solutes in plasma, and thus are also taken out of solution. Either way, the blood pH is being buffered to remain within its normal narrow range of pH.

Figure 15.27 The events occurring when carbon dioxide enters a erythrocyte include the formation of carbonic acid and the resulting buffering by haemoglobin and plasma proteins.

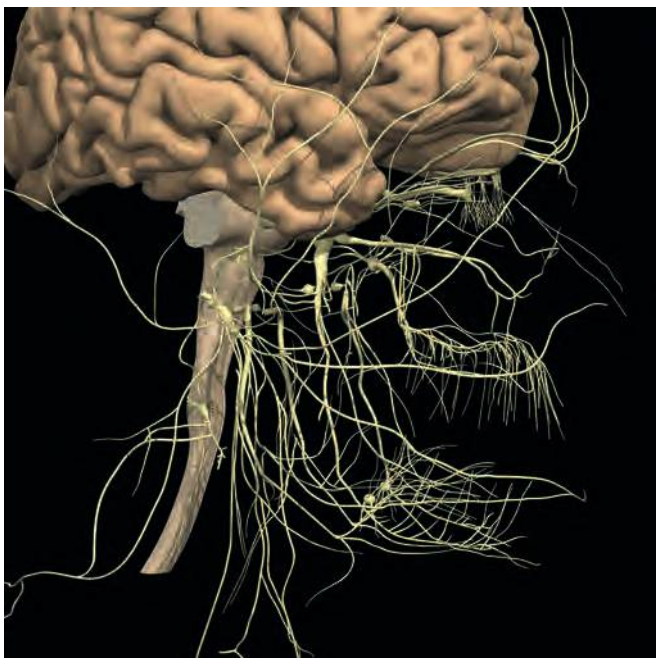


The rate of ventilation is controlled by the respiratory control centre in the medulla oblongata

The use of skeletal muscle demands the use of ATP molecules, and thus an increase in the rate of aerobic cellular respiration. Active muscle tissue consumes much more oxygen and produces much more carbon dioxide than muscle tissue at rest. The body must have a mechanism to ensure that the rate of transport of these respiratory gases meets the needs of the increased demand. One specific requirement under these conditions is an increase in the rate of breathing, the ventilation rate.

The ventilation rate is under the control of an area of the medulla oblongata of the brainstem. This area of the medulla is known as the respiratory control centre and has two mechanisms that come into play when the rate of ventilation needs to increase.

- Receptor cells, known as chemosensors (or chemoreceptors), located in the inner wall of the aorta and carotid arteries, detect when there is an increase in carbon dioxide level and the associated decrease in blood pH. When stimulated, these receptors send action potentials to the medulla's breathing centre.
- The medulla itself contains the same kind of chemosensors. As the blood passes through the capillary beds located in the medulla, increased carbon dioxide levels and decreased pH are detected.



Nothing more than the brain and cranial nerves emerging from the brainstem are shown in this illustration. If you follow where the nerve branches are from the upper jaw straight to the left, you will find the medulla oblongata of the brainstem. The cranial nerve branches include not only facial sensory and motor nerve impulses, but also the autonomic nervous system control of heart rate and ventilation rate. Notice that the cranial nerves going straight down out of the picture are heading towards the chest and abdomen.

The homeostatic blood pH is 7.35 to 7.45, which is very slightly alkaline. The low end of this pH range is because of the dissociation of carbonic acid explained in the previous section. Under normal circumstances, buffering by haemoglobin and plasma proteins prevents any change in pH. But when you are exercising heavily, the buffering mechanisms are overtaxed and the excess hydrogen ions lower the blood pH to the low side of normal.

To increase the ventilation rate, the medulla's respiratory control centre sends action potentials to the diaphragm, intercostal muscles (the muscles between the ribs), and muscles in the abdomen. The mechanism of breathing is not altered, just the frequency. When the physical exertion ceases, or at least decreases, the

chemoreceptors detect the decrease in carbon dioxide level in the bloodstream, or the corresponding slight increase in blood plasma pH, leading to a decrease in ventilation rate.

Living and breathing at high altitude

There is a common misconception that the air at high altitudes contains less oxygen by percentage than the air at sea level. This is not true: the percentage of gases in the air does not change as altitude increases, it is the air pressure that changes. Air at higher altitudes is at a lower pressure. This means that all the molecules in the mixture are more spread out than in a mixture at sea level. When you breathe less dense air, diffusion of oxygen across the alveoli into the bloodstream is less efficient, and less oxygen enters your bloodstream.

When someone first arrives at a high altitude, physical activity can lead to almost immediate fatigue. Other high-attitude symptoms can include vision problems, nausea, an abnormally high pulse rate, and difficulty in thinking clearly. These symptoms are often called altitude sickness or mountain sickness. Severe cases of altitude sickness can lead to fluid accumulating around the brain or in the lungs, and can become life-threatening. A person suffering from severe altitude sickness should return to a lower altitude as soon as possible.

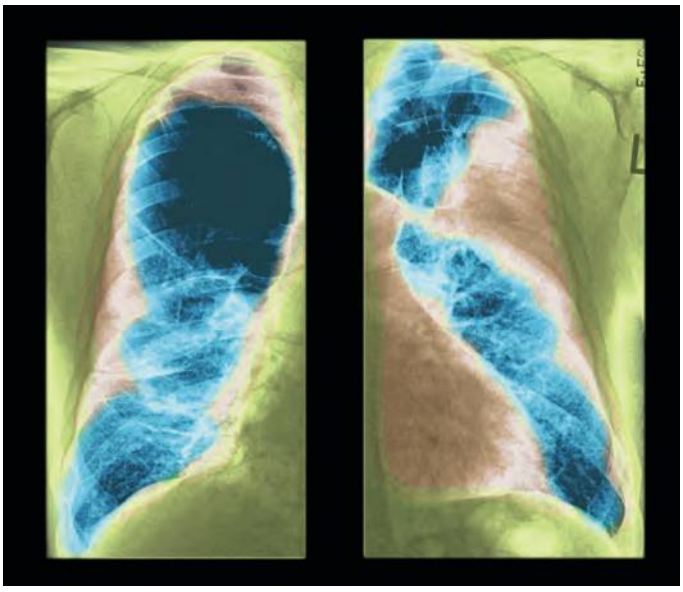
On arrival at high altitudes, our bodies attempt to compensate by increasing the ventilation rate and heart rate. This is stressful for the body and is not a long-term solution or adaptation. Over time, acclimatization does occur. Some of the physiological responses involved in acclimatization are:

- an increase in the number of erythrocytes and amount of haemoglobin
- an increase in the capillaries in both the lungs and muscles
- an increase in lung size and surface area for oxygen and carbon dioxide exchange
- an increase in myoglobin within muscle tissue.

Training for some sports is often done at high-altitude locations in order to take advantage of some of the possible acclimatization adaptations, such as increased haemoglobin and numbers of erythrocytes.

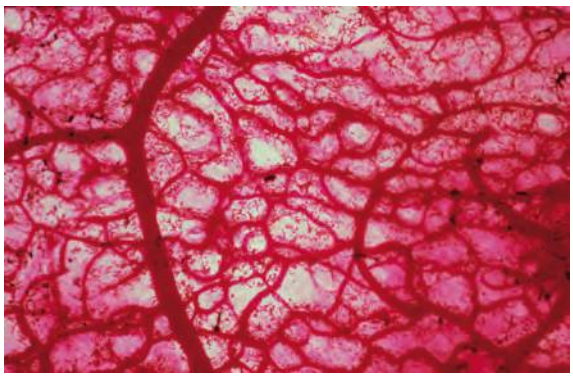
Mountaineers who climb high peaks such as Mount Everest typically set up several base camps at increasing altitudes. They spend time at each base camp in order to allow a certain degree of physiological adaptation at each new, higher, altitude.

Causes and treatments of emphysema



A colour-enhanced frontal X-ray of a patient with emphysema. The areas in blue are diseased with emphysema and there is a large infected diseased cavity in the right lung (left side of photo).

Earlier you learned about the internal workings of lung tissue (Section 6.4). As you recall, the surface area where oxygen and carbon dioxide is exchanged is between the small air sacs (alveoli) and capillary beds. Emphysema is a disease where many of the alveoli have become severely damaged, with gaping holes left where healthy tissue once existed. The majority of people suffering from emphysema were (and sometimes still are) cigarette smokers for many years. Emphysema is often accompanied by other damage to the airways leading to the alveoli, and collectively the symptoms are referred to chronic obstructive pulmonary disease (COPD).



Capillaries and alveoli are extremely thin and delicate tissues consisting of single cell layers. When emphysema destroys an area of a lung, there are no remaining healthy cells to regenerate the dead tissue. This photo shows small delicate blood vessels, including capillaries.

NATURE OF SCIENCE

In many countries, people are much more informed about the dangers of smoking compared with a few decades ago. This is in large part because of the role of scientific research into the damage done by smoking.

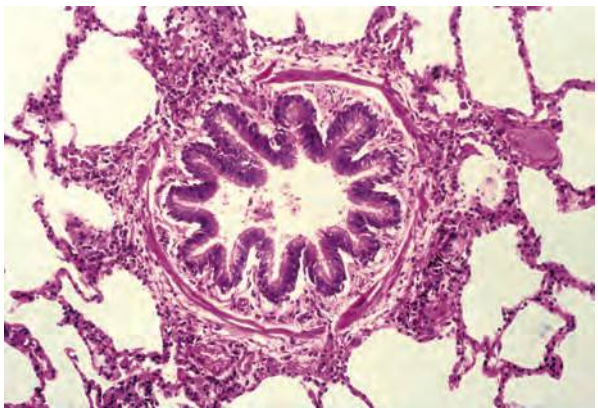
In addition to smoking cigarettes, long-term exposure to the following is known to cause or exacerbate the symptoms of emphysema:

- marijuana smoking
- exposure to second-hand smoke of cigarettes or marijuana
- exposure to air pollution
- exposure to manufacturing fumes
- exposure to coal and silica dust.

The incidence of cigarette smoking is decreasing in many countries, especially in those countries where public opinion has been informed by scientific research showing the harm that smoking does. However, there are some countries where the incidence of smoking has rapidly increased over the last two decades.

If the exposure to smoke or other agents leading to lung damage is stopped soon enough, the lung tissue can fully or partially repair itself. However, most people who are diagnosed with emphysema have lung damage that is far too severe for self-repair. Treatments are centred around slowing further damage by stopping the exposure to the causative agent(s) and medications that help relieve some of the symptoms. Eventually many people with emphysema have to take supplemental oxygen from a container, administered through small tubes into the nostrils.

Identification of lung tissues with light and electron micrographs



A light micrograph of a bronchiole and many alveoli within a lung. The bronchiole is shown in section (with many invaginations) and all of the surrounding (somewhat) circular areas are sectioned alveoli.

The functional tissue of lungs, the alveoli, and capillaries are best viewed under an electron microscope. Below you can see an illustration of a capillary (in section) (running in/out of the page). Inside the capillary are portions of three erythrocytes also cut in section. On either side of the capillary are portions of two alveoli, one on the left, one on right. Only the portion of the alveolar membranes closest to the capillary is shown, as each alveolus is too large to show in full. The nucleus of an alveolar cell is shown just to the right of the three erythrocytes.

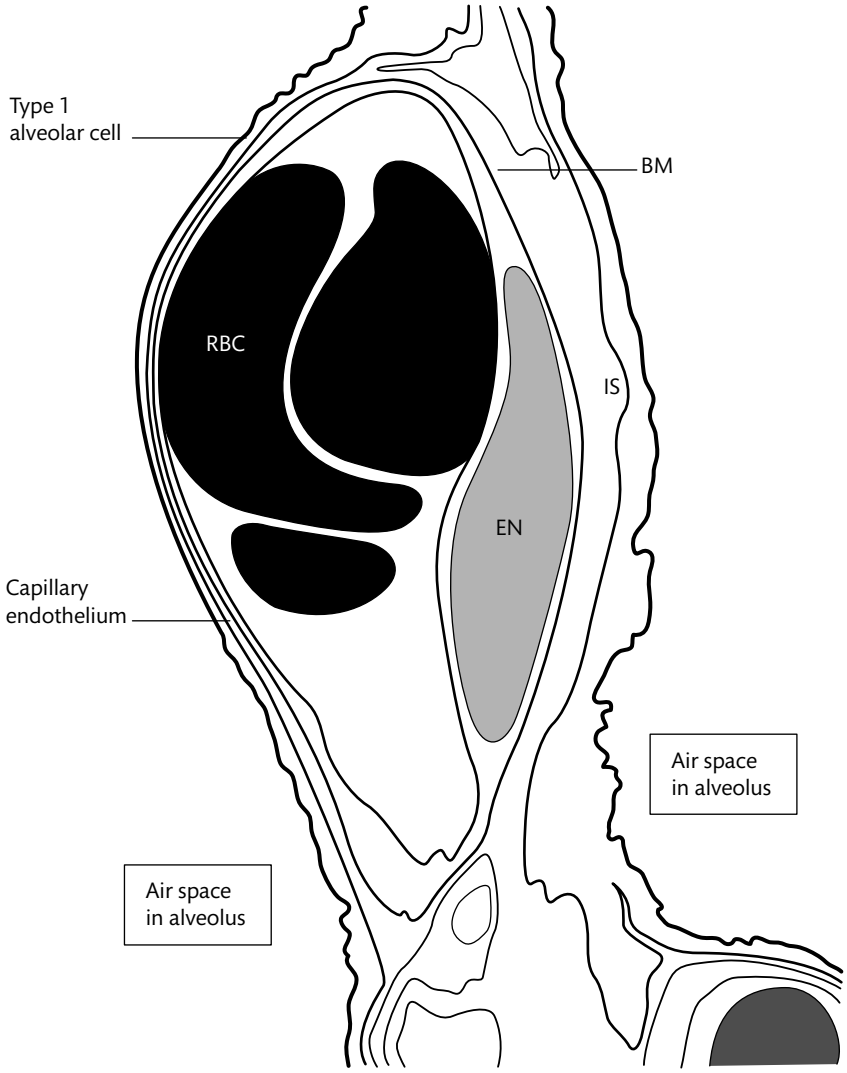


Figure 15.28 A capillary with two adjoining alveoli. N, nucleus; RBC, red blood cell (erythrocyte). http://www.78stepshealth.us/human-physiology/structure-of-the-respiratory-system.html

CHALLENGE YOURSELF

3 Using the artwork of a small area of lung tissue shown on the left, answer the following questions.

(a) Why do the erythrocytes shown not have the characteristic shape of a bi-concave disc?

(b) How many cell membranes would an oxygen molecule have to pass through in order to diffuse from the alveolar air into an erythrocyte?

(c) In what phase of the cell cycle is the alveolar cell showing a nucleus? Give evidence.

(d) In the lower right hand corner is a dark shape. What do you interpret this dark shape to be?

Exercises

21 Explain why adult myoglobin and foetal haemoglobin need to have a greater affinity for oxygen compared with adult haemoglobin.

22 What physiological advantage does the Bohr shift provide?

23 Why does the pH of blood lower when you are active, for example when you are exercising?

24 Why would it be inaccurate to say that heavy exercise makes the blood more acidic?

25 Why do you not have to think consciously about breathing faster when you are physically active?

Practice Questions

1 **(a)** Define the term nutrient. (1)

(b) Discuss the relationship between nutrition and rickets. (3)

(*Total 4 marks*)

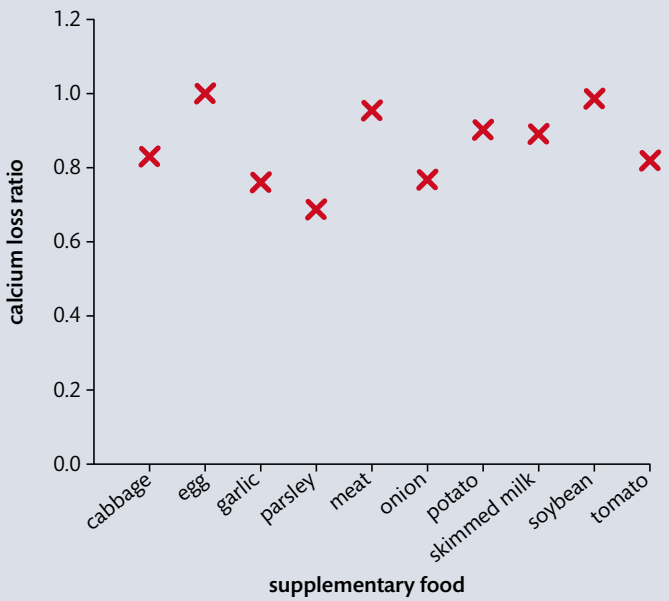
2 Explain the Bohr shift of an oxygen dissociation curve during gas exchange.

(*Total 6 marks*)

3 Osteoporosis is a major health problem for many post-menopausal women. As the ovaries reduce their secretion of oestrogen, calcium is gradually lost from bones, weakening them and increasing the chance of fractures. To test whether diet influences the rate of calcium loss, ovaries were removed from groups of female rats and the rats were then either fed a control diet or the same diet with 1 g of a supplementary food per day. The rate at which the rats excreted calcium was measured. The ratio of calcium loss between the control rats and the rats that were given a supplementary food was calculated.

$$\left(Ratio = \frac{\text{loss with supplementary food}}{\text{loss in control rats}}\right)$$

The results are shown in the graph below.



Nutrition: Effect of vegetables on bone metabolism *Nature*, 401, 23 September, pp. 343–344 (Roman C, Muhlbauer and Feng Li 1999), Copyright 1999. Reprinted by permission from Macmillan Publishers Ltd

(a) **(i)** Identify which supplementary food was most effective in reducing calcium loss. (1)

(ii) Identify which supplementary food was least effective in reducing calcium loss. (1)

(b) Among the ten foods shown in the graph, seven are plant products (vegetables) and three are animal products. Discuss whether the plant or the animal products were more effective at reducing calcium loss. (3)

(c) Suggest a trial, based on the results shown in the graph, that could be done to try to reduce osteoporosis in humans. (3)

(*Total 8 marks*)

4 A major requirement of the body is to eliminate carbon dioxide (CO_2). In the body, carbon dioxide exists in three forms: dissolved CO_2, bound as the bicarbonate ion, and bound to proteins (e.g. haemoglobin in red blood cells or plasma proteins). The relative contribution of each of these forms to overall CO_2 transport varies considerably depending on activity, as shown in the table below.

CO_2 Transport in blood plasma at rest and during exercise

Form of transport	*Arterial*	Rest *Venous*	Exercise *Venous*
	mmol l^{-1} blood	mmol l^{-1} blood	mmol l^{-1} blood
Dissolved CO_2	0.68	0.78	1.32
Bicarbonate ion	13.52	14.51	14.66
CO_2 bound to protein	0.3	0.3	0.24
Total CO_2 in plasma	14.50	15.59	16.22
pH of blood	7.4	7.37	7.14

(a) Calculate the percentage of CO_2 found as bicarbonate ions in the plasma of venous blood at rest. (1)

(b) (i) Compare the changes in total CO_2 content in the venous plasma due to exercise. (1)

(ii) Identify which form of CO_2 transport shows the greatest increase due to exercise. (1)

(c) Explain the pH differences shown in the data. (3)

(*Total 6 marks*)

5 Describe the process of erythrocyte and haemoglobin breakdown in the liver. (4)

(*Total 4 marks*)

Theory of knowledge

An astronomer, a physicist, and a mathematician are on a train going to a conference in Edinburgh. Out of the window, they see a solitary black sheep.

Astronomer: That's interesting, sheep in Scotland are black.

Physicist: It would be more prudent to say that *some* of the sheep in Scotland are black.

Mathematician: To be more precise, we can say that in Scotland there exists at least one field in which there is at least one sheep, which is black on at least one side.

What does this story reveal about scientific observations, hypotheses, and conclusions? What does it reveal about the nature of each of the disciplines represented?

Is biology less exact than physics or mathematics? If there had been a biologist on board the train, what would he or she have said about the sheep?

What is this chapter all about?

This chapter has some ideas, quotes, anecdotes, case studies, and many unanswered questions, but very little factual information. Why? Because with Theory of knowledge (TOK), you are the knower. This concept should stimulate your brain. It should be exciting to think that you are the expert. You have had a decade or more of formal education, and even more years of life experience, giving you ideas in the form of knowledge, beliefs, and opinions.

You are the knower.

On the other hand, it is a bit intimidating to think that there are some things that no one will ever know the answer to. You are encouraged to explore, develop, and share your views, as well as actively seek the views of your classmates.

On the right track?

How will you know if you are answering TOK questions in the 'right' way, as the answers are not given in this book or by your teacher? Here are two guidelines to consider.

- If you think the question has a quick, simple answer, such as 'yes' or 'no', you can be pretty sure that you are not treating it like a TOK question. If you think the answer has many sides to it, is a debatable area, or leads to further questions, you are probably on the right track.
- Ask yourself, 'Am I pushing myself a little bit outside my comfort zone and exploring other ways of seeing an issue?' If so, you are on your way to scratching through the surface and getting to the interesting issues. That is the stuff of intellectual stimulation and growth. That is one of the challenges of the IB programme in general and TOK in particular.

Debates

To get your brain warmed up, consider the following two statements about the nature of all human beings on Earth.

A: We are all the same.

B: We are all different.

Use your biological knowledge to support or refute these two claims. Choose one, and try to imagine someone saying to you, 'That's not true! How can you say that?' How would you respond to that person? (Keep it polite, of course.)

Now try these two statements.

X: Biology is a collection of facts about nature.

Y: Biology is a system of exploring the natural world.

Use your critical thinking. Critical thinking is characterized by reflective inquiry, analysis, and judgment. Ask yourself, 'Should I believe this?' 'Am I on the right track?' 'How reliable is this information?' In short, you are deciding whether or not you should accept something as valid or not. Again, if you think it is an easy, quick decision, then you are not treating the question in the way that you should.

Critical thinking: Is this statement valid? What is its source? Is the person who said it a reliable person? Do they have a bias that I should know about? Bias is a good term to know in TOK. It refers to a type of prejudice whereby a person gives an unfair preference to one opinion over another, rather than giving a balanced argument.

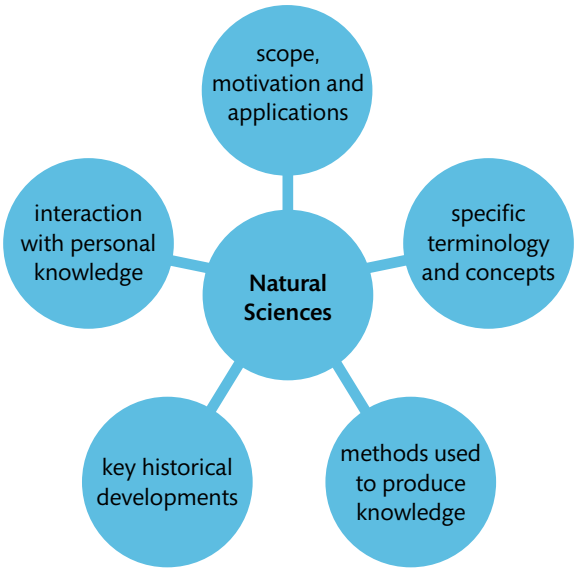
More debates

Coming back to the pairs of statements above, what would lead someone to believe one or the other statement? In each pair, could it be possible that both statements are valid? Or are they mutually exclusive? What about the following two statements?

- There is only one scientific method that is universal throughout the world: only by following the same method can scientists reach the same results and conclusions.
- Different scientists and different cultures in different regions of the world use different versions of the scientific method to obtain valid results and conclusions.

The TOK framework

One of the most important skills students are asked to develop in the IB is analysis. The TOK framework is a useful tool for analysing knowledge issues. When analysing a particular scenario, students are not asked



A knowledge framework applied to the area of knowledge of the natural sciences.

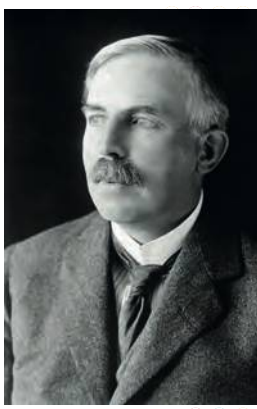
to address each of the five points below exhaustively, but this framework can be a good place to start.

Try it out on the example of the scientists on the train looking at the sheep. There may be some aspects of the framework that apply nicely to the sheep example, and others that do not fit well, but give it a try. Throughout this chapter there are a certain number of case studies; as good practice, it is worth analysing some of them based on the knowledge framework.

Nature of science(s)

> "All science is either physics or stamp collecting."
> Ernest Rutherford

What did Rutherford mean by the above? Is he justified in saying that chemistry, biology, or other branches of science, are only there to catalogue and classify phenomena in nature? What does his statement imply about the degree of prestige or respect each scientific discipline enjoys? Is it possible to understand all sciences by studying one of them?



Ernest Rutherford was a physicist: you probably identified the bias he had from his quote!

What is knowledge?

Below are some knowledge issues/knowledge questions to consider.

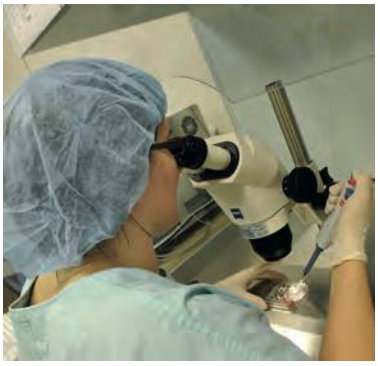
- What counts as knowledge in biology?
- How does biological knowledge grow?
- What are the limits of knowledge?
- Who owns knowledge?
- What is the value of knowledge?
- What are the implications of having or not having knowledge?
- Is there one way that is best for acquiring knowledge?
- Where is knowledge? Is it a 'thing' that resides somewhere: is it in books, in your head, in a computer database?

Look at the following images. Use the list of questions above and your critical thinking to evaluate whether some or all of these are valid as scientific knowledge. For example, does mythology count as scientific knowledge?


Mythology


Electronically stored data


A biology diploma


Experimental work

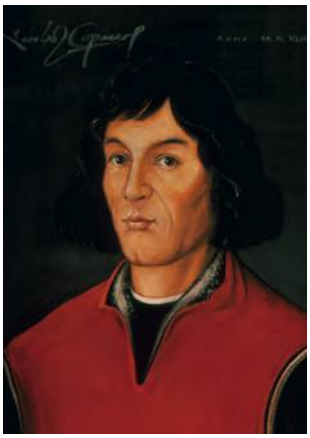

Ancient belief systems


Websites and email systems


Student discussions


Religious texts


Libraries



Copernicus is the scientist who mathematically showed that the Earth goes around the Sun, and not the other way round. This is an example of a paradigm shift, another good term to know in TOK. A paradigm shift is a fundamental change in the way we see or understand something.

> "To know that we know what we know, and to know that we do not know what we do not know, that is true knowledge."
> Copernicus

How do we know?

Here is an example of scientific knowledge in biology: 'The organelle in a plant cell that is responsible for photosynthesis is the chloroplast'.

How could you verify that? How can you be sure that there is not another part of the cell that photosynthesizes? Is it a falsifiable idea? Such questions are second-order questions. They are not about the thing we want to know, they are about how we know it. Such knowledge questions are on a different level.

Epistemology is the study of the theory of knowledge and it raises the question: 'How do we know what we know?'

Case study 1 Life on Mars?

This is not a simple question. Despite several visits by space probes, no conclusive evidence has been discovered on Mars that can lead scientists to declare that there is life on its surface. And yet the search continues. The most compelling evidence that there was once life on Mars comes from a meteorite found in Antarctica that The National Aeronautics and Space Administration (NASA) claims came from Mars and contains fossils of bacteria.

If you apply your critical thinking to this, some knowledge questions should pop into your mind. How do we know that this chunk of rock is really from Mars? How did it get to Earth? How does NASA know that the 'fossils' are from bacteria? Could they have been formed from non-living chemical reactions? How important are such discoveries in ensuring funding for future missions?

Is NASA planning to collect more fossils from Mars directly, and bring them back to Earth to study? Could there still be colonies of bacteria living on Mars today, or is life extinct on the red planet?

From this specific example, two more general questions arise. Is it possible to really 'know' the truth? Is information absolute or relative?

Are you an empiricist or a rationalist?

Empiricism = the belief that our senses allow us to acquire knowledge. Rationalism = the belief that reason allows us to acquire knowledge.

Be careful: critical thinking does not mean you criticize everything. It means you are aware of questions of validity. You are not being negative, you are just being inquisitive and prudent.

Ways of knowing

In the list of TOK ways of knowing listed to the right, are there some that are better suited to the natural sciences than others? Are there any that can be completely eliminated from the list when dealing with the natural sciences? What about ones that are absolutely necessary?

Ways of knowing:

- language
- sense
- perception
- emotion
- reason
- imagination
- faith
- intuition
- memory.

Case study 2 Babies born on a full moon

Ask an experienced midwife 'Are more babies born on a night when there is a full moon?' and chances are pretty good she will say yes. You would have no reason to challenge her: she is the expert. She is an eyewitness to this phenomenon.

But knowledge questions arise. Where is she getting her information? How does she know? Is it just a feeling, an intuition, a belief? Or is this knowledge claim based on carefully analysed statistics comparing birth numbers with a lunar calendar?

As it turns out, the statistics do not support this knowledge claim. The evidence from maternity ward numbers does not show a correlation between births and the full moon. So what is going on? Is the midwife lying? The chances are she is the victim of something we all are susceptible to: confirmation bias. Confirmation bias happens when we only remember the times when something confirmed our beliefs, and ignore the times when something refuted our beliefs. In the case of the midwife, on a busy night she might look out the window, see a full moon, and cry out to her colleagues, 'See? I was right! More babies on nights when there is a full moon.' Two weeks later, on another busy night, she looks out the window and what does she see? No moon at all because, it's the new moon. It is unlikely that she will now go around to all her colleagues and say 'Sorry, I was wrong: it's a busy night and yet there is no full moon.' It is more likely that she will forget this negative result and only remember the positive results, thereby showing a bias for confirmation.

As an afterthought: should we tell her she's wrong? It could be argued that she's not hurting anyone and that it's lots of fun to have these sayings in our culture. Having shared beliefs unites people and strengthens a sense of community and belonging. Is it better to be right or to belong?

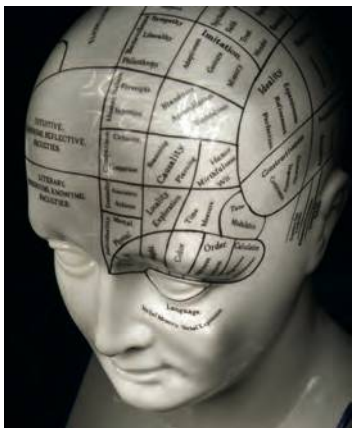
Catching a cold

Despite the biological evidence that colds are caused by viral infections, many people believe that you can catch cold from being exposed to low temperatures or changes in humidity.

Who is right? Where does the truth lie? For something to be considered 'true', does it have to be formally proven using a scientific method? Is a profound conviction that something is true good enough to make it valid? If one person believes that something is true, does that make it true or does there have to be a certain number of believers before the idea can be considered true?

Phrenology

The pseudo-science of phrenology claimed that the shape of a person's skull, and the bumps and indentations on it, determined a person's intelligence, personality, and talents. More controversially, it was used by some to justify the superiority or inferiority of 'races' of humans.



How do you think it was demonstrated that the 'laws' of phrenology were not, after all, scientifically valid?

Tongue map

As students and teachers, what do we claim to know about biology? Are we justified in making such claims? How?

What experiences have you had that give you insight concerning these issues? Consider the following example.

For decades, the idea of a 'tongue map' (i.e. certain zones of the tongue relate to certain tastes) was propagated by biology textbooks, and taste-test investigations were suggested as lab work for students. It has since been shown that all parts of the tongue can taste sweet, sour, bitter, and salty.

> "There must be no barriers for freedom of inquiry. There is no place for dogma in science. The scientist is free, and must be free to ask any question, to doubt any assertion, to seek for any evidence, to correct any errors."
>
> Robert Oppenheimer
> Barnett 1949

Art and imagination

Is there a place for imagination and creativity in science? Are there any parallels between biology and art? Could it be argued that just as an artist sees things in his or her own way, so a scientist sees things in his or her own way? Or, on the contrary, are science and art diametrically opposed ways of interpreting nature?

Case study 3 Spirit/soul

In 1907, Dr Duncan MacDougall conducted experiments to determine whether or not people lost mass after death. His results seemed to suggest that they did, and led him to the conclusion that the human soul weighed 21 g. Since his experiments (some of which did not give conclusive results) were done with scales of questionable accuracy and he had only six subjects, his conclusions are widely criticized and are not taken seriously by the scientific community today.

Will questions about souls always remain beyond the capabilities of science to investigate or verify? Why hasn't anyone repeated this experiment in over a century? What do you think the reaction of the religious community would be if scientists repeated MacDougall's experiment?

Decisions, decisions …

Should experiments be performed to answer fundamental questions, or should they only be done if they have a useful application in our everyday lives?

Who should decide which research pursuits are of the most value? Who should decide on how funding is distributed, or the prioritizing of the use of laboratory space and resources? Universities? Governments? Committees of scientists? Taxpayers?

Should research about a tropical disease such as malaria be paid for by tax money from non-tropical countries?

Is there an end?

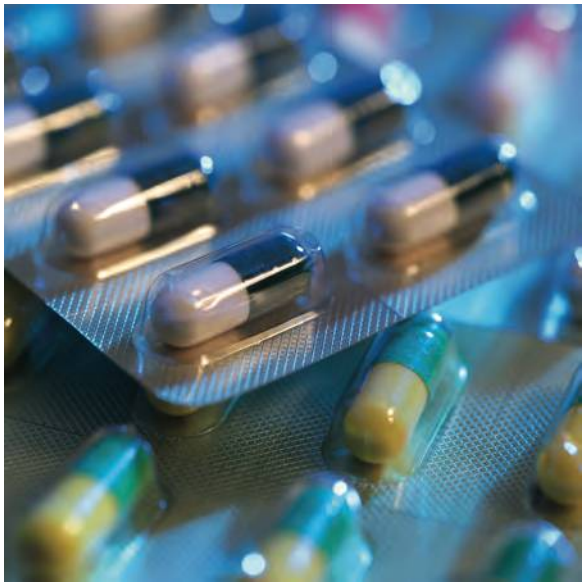
Is scientific knowledge progressive? Has it always grown? Imagine a graph with scientific knowledge on the *y*-axis and time on the *x*-axis. How would you draw the graph? Would it be a curve or would it be linear? Is it always increasing? What units would you use? Could the graph ever go down: in other words could scientific knowledge ever be lost (maybe because of war, a laboratory burns down, a famous scientist dies)?

Could there ever be an end to science? If there was an end, what would be the consequences?

> "Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world."
> Louis Pasteur

Doctor, which drug treatment is best for me?

How do doctors know which medication is the best for their patients? One way is for them to keep up with the latest breakthroughs and developments published in scientific and medical journals. Doctors put their faith in these prestigious peer-reviewed journals and, because they do not have the time or the budget to do all the clinical trials themselves, they trust that the researchers doing the work are following sound practice. The problem is, a large percentage of the studies are being funded by the companies who make the drugs and, according to epidemiologist Dr Ben Goldacre's 2012 book *Bad Pharma*, it is common practice in the pharmaceutical industry to use a wide variety of tricks and manipulations to make a new drug look good in clinical trials. One trick goes something like this: a company will set up a 2-year trial to test a new drug and then, after only 6 months, it decides to stop the trial and publish the data because the numbers show that its drug is performing well. This is advantageous to the company because it saves money (trials are very costly), and it reduces the chances that participants develop side-effects or show negative results after 6 months. Doctors reading about the clinical trials will never be informed, however, that the trials were stopped early. Another trick is to not report in the published study any participants who dropped out of the trial because they felt ill from side-effects. That way, by only mentioning the people who stayed in the study, they can report that, at the end of the trial, none of the participants complained of any major side-effects. In short, Goldacre says that the studies being published are not showing all the data and that, in order for doctors to decide whether a drug is safe to prescribe, they need to see both the positive and the negative results. What knowledge issues does Goldacre's book raise about the highly competitive $600 billion pharmaceutical industry? Do the practices he denounces sound like the kinds of things your biology teacher encourages you to do in your lab investigations? If you were a scientist working at one of these companies, and you decided to complain and point out that some of the trials seemed unfair, what do you think your boss's reaction would be? If a company decided to publish the positive as well as the negative results of its drug trials, what do you think might happen to the sales of their drugs? Lastly, as doctors find out more and more of the practices exposed, what will happen to their faith in the data presented by the medical journals? What kind of critical thinking or TOK questions should they pose when they pick up a medical journal and read about the latest breakthroughs in drug research?



The placebo effect

One of the ways that scientists test a drug is to compare it with a placebo. A placebo is a pill that contains no active ingredients: it is often just a sugar pill. To find out if a new drug is effective, one group of volunteers in a study is given the new drug and another group is given a placebo. Neither group knows whether it is taking an active pill or a placebo. Surprisingly, even in the group taking the placebo, there are usually patients who report that they feel better. This is called the placebo effect.

Researchers studying the placebo effect have observed that the following things have a positive influence on how effective the patients thought the pill was:

- the doctor was wearing a white lab coat
- there were diplomas on the wall in the doctor's office
- the doctor sat down and listened attentively to the patient.

The medical community is essentially unanimous on the validity and power of the placebo effect, and yet the mechanism of how it works is poorly understood. For example, astounding as it may sound, placebos seem to have an effect even when people are told that they are receiving a placebo. Some participants still feel better, even when they are conscious that they have not been given any active drugs.

Inhabitants of industrialized countries often scoff at tales of traditional medicine men and healers in indigenous peoples. And yet, those same critics may very well accept the effectiveness of the placebo effect: an effect that appears to be produced essentially by ritual. What knowledge issues are raised by this puzzling effect? What does it say about the limits of modern medicine?

Models

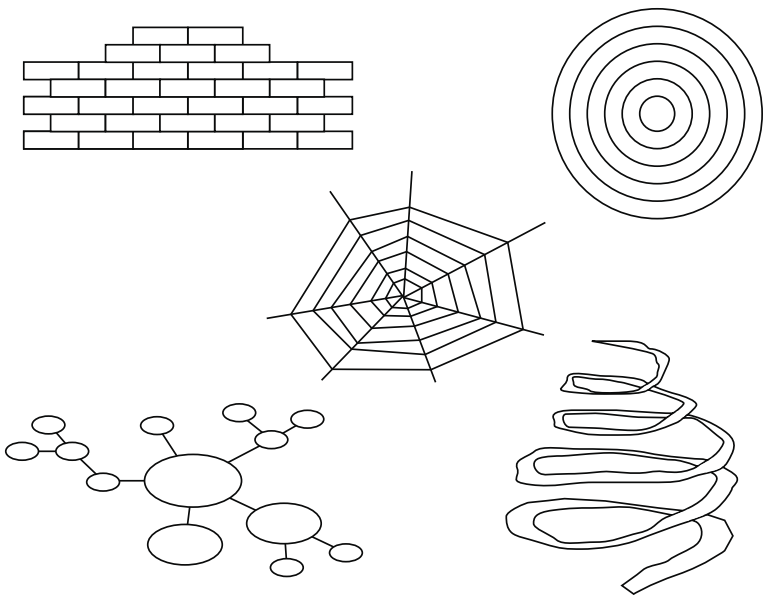
The double-helix shape for DNA, and the fluid mosaic membrane model, are examples of models that were created in order to explain observed phenomena. Are such models just inventions? If so, how is it that they can be used to make predictions or explain natural phenomena?

Look at the false-colour electron micrograph (above right). The magnification and resolution are not good enough to see how the integral or transmembrane proteins and cholesterol are arranged in the membrane, but chemical tests reveal that they are there. This is why the fluid mosaic model was introduced. It is a proposed explanation for how the various components of the membrane are arranged. If the model successfully fits the observed phenomena, does that validate it as being true?

The double line in the centre of this photomicrograph shows the phospholipid bilayer of a membrane.

> "All models are wrong, but some are useful."
>
> George E. P. Box (innovator in statistical analysis)
>
> Box and Draper, 1987. Copyright © 1987 by John Wiley & Sons, Inc.

Which of the following conceptual drawings best represent the interconnections between the ways of knowing and areas of knowledge? In what ways might these metaphors be useful?



Who's right?

Among all the points of view that are available to you in the classroom, at home, in the media, on websites, how do you know which one is right?

Religion in an age of science

In what ways could someone's cultural or religious background influence his or her acceptance of certain scientific theories?

There was a time when scientists hesitated to publish their works out of fear of the church. Have the tables turned? Are there religious writers who fear scientific criticism if they publish their ideas?

If a student writes on an IB exam that he or she refuses to answer the questions about evolution by natural selection because of his or her religious beliefs, should he or she get any marks?

In 1663, the Roman Inquisition condemned Galileo for defending the idea that the planet Earth goes around the sun and he remained imprisoned for nearly a decade before he died. In 2010, the Catholic Church formally apologized for Galileo's condemnation.

Ockham's razor

Simply put, the principle of Ockham's razor states that, all other things being equal, the simplest explanation should be preferred. This is reflected in the idea of parsimony: seeking out the least convoluted solution. Scientists take this principle very seriously and yet some aspects of science seem to be extremely complex. Is there a conflict here?

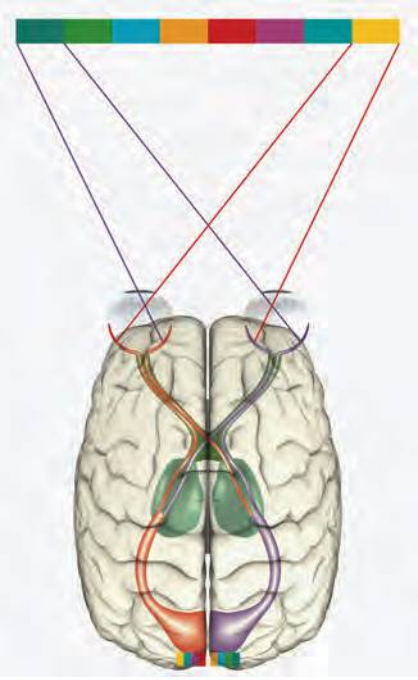
Limits of perception

> "You cannot speak of the ocean to a well frog ..."
>
> Chuang Tzu Taoist text (written more than 2000 years ago)

Can we here on Earth possibly know of worlds beyond our own? Can we possibly know what the distant past was like, or what the distant future will hold? Or are we like a frog at the bottom of a well trying to understand what the ocean might be like?

The eye is not a camera

A fun activity to do in a classroom is to have someone unknown to the students barge in during a lesson, say something, take something off the teacher's desk, and leave, after which the teacher asks each student to take out a sheet of paper and write down a description of the person, and what he or she said and did. The students' observations are often astounding in their diversity, and the activity demonstrates how human perception is notoriously bad at picking up crucial details, and notoriously good at filling in missing information. 'The eye is not a camera' is a good example of a knowledge claim that can be explored and discussed after such an observation activity.



Although we associate the eyes with vision, truly seeing something means interpreting the signals that arrive at photoreceptors in the eyes. This interpretation is done in the brain, so in fact, do we see with our eyes or our brain?

Another knowledge claim on this theme also relates to eyewitnesses: 'all memories are reconstructed memories'. Have you ever had a story in your family that was told time and time again for years, and then one day you found out that the event in question never actually happened? And yet, you could swear that you can remember the event clearly. Or have you ever watched a video recording of something you

experienced and thought to yourself, 'That's funny, I don't remember it being like that: my memory of that event is very different'. Such examples put into question the validity of eyewitnesses' testimonies in a court of law. Given that we ourselves know that our memories and observations can trick us, should we trust an eyewitness's account as irrefutable evidence during a trial? Are such testimonies reliable enough to put defendants in jail or to sentence them to death? This theme is explored well in Syndey Lumet's 1957 film *Twelve Angry Men*.

We were wrong, here's the real story …

In palaeontology, it seems that every time a new hominid fossil is dug up, we have to redraw the human family tree. If you search the internet for human phylogeny, you will probably find that few sources agree with each other. When are they ever going to make up their minds and get it right? Likewise, regarding questions of diet and nutrition, every few years nutrition experts change their minds about dietary advice.

Does this frequent revision give credibility to science, or does this make science less credible?

Argument for the question: It's important for scientists to be able to modify ideas as new evidence is revealed. This is how science grows and progresses and, without such a system, we would be intellectually stuck.

Argument against the question: Why can't these so-called experts make up their minds? One year they say one thing, and then a year or two later they say 'Oh, we were wrong, here's the real story.'

Archaeopteryx

Archaeopteryx is one of the most famous fossils in the world. It has some features of a dinosaur, such as reptile teeth and a bony tail, but it also has some bone structures like a bird and it has the most bird-like feature of all: feathers. It did not take long for some observers to jump to the conclusion that Archaeopteryx is the 'missing link' between dinosaurs and birds. Can we be so sure that this fossil is the transition between the two? Are physical features enough to base such a decision on? What kind of evidence would give more credibility to this claim?

Many palaeontologists shy away from terms like 'missing link': what features of this use of language make the term unscientific?

NATURE OF SCIENCE

For centuries, it was firmly believed that rats, maggots, and mould sprang from rotting meat and vegetable matter. This was called spontaneous generation. It took tireless experiments by Louis Pasteur and others to refute this idea and prove that the rats, maggots, and mould came from the surrounding environment.

The end of spontaneous generation

The idea of spontaneous generation has been shelved as unscientific. It has no value as biological knowledge, but it does have historical value and it helps to illustrate how science works.

This is a good example of an original hypothesis that was disproved and falsified by experimentation. It can be argued that, in order for something to be considered valid as scientific knowledge, it has to be verifiable. If experiments show that the results do not support the hypothesis or even refute it, the

idea is falsified. This assumes that the experiment is repeatable. Other scientists should be able to do the same experiment and get similar results. Imagine the consequences of the following situation.

The colleague of a famous scientist dies unexpectedly and a student of his decides to publish extracts from the laboratory notebooks. The notes are filled with interesting ideas but also contain severe criticisms of the methods of the famous scientist. For example, only the experiments that gave evidence supporting the famous man's hypotheses were considered and the others that refuted the hypotheses were ignored. This goes against everything the scientific method is supposed to represent. This scenario happened when Claude Bernard died in 1878. He had been working with Louis Pasteur and it took a great amount of persuasion and force of personality for Pasteur to save his reputation. He had lots of both.

Case study 4 Science and government

Trofim Denisovich Lysenko was a Soviet biologist who opposed the ideas of Mendel and Morgan concerning genetics. Instead, he promoted the idea that acquired characteristics could be passed on from one generation to the next. Under Stalin, he was promoted to a high-ranking post in agronomy and given his own scientific journal for publishing his ideas. The agricultural techniques he developed were used to feed the Soviet population and the Red Army. Once Stalin and Khrushchev were no longer in power, however, his methods were widely criticized and his theories attacked for lack of scientific validity. An inquiry revealed that, in order to retain his powerful position and promote his ideas, he had intimidated and removed scientists who questioned his theories. He was finally fired from his post at the Institute of Genetics in 1965 and his reputation was crushed. What does this story reveal about the influence of politics on scientific theories? In what ways does it reveal scientific bias? How do we know that Lysenko's critics were not simply trying to push their own opposing political agenda?

Unprovable assumptions?

Does biology make any assumptions that are impossible to prove? Consider this: all events in nature are caused by physical phenomena.

In other words, every natural event can be explained by the interactions between atoms and molecules. Is such a statement provable? If we find enough examples of instances where this is true, can we proceed by induction that it is true for all phenomena? This seems reasonable, and yet the philosopher David Hume criticized induction, saying that there is no logical reason to assume that it is the case. Consider Karl Popper's quote about swans, which illustrates clearly the problem of induction.

> "No matter how many instances of white swans we may have observed, this does not justify the conclusion that all swans are white."
>
> Karl Popper
>
> Popper 1992a

Case study 5 Choosing a boy or a girl

A clinic claims that it has developed a new technique for filtering sperm cells in such a way that future parents can choose whether to have a boy or a girl. The doctors at the clinic claim they have a 95% success rate, which is considerably higher than current sperm separation techniques. In an effort to protect the secret technique from being stolen by competing clinics, the staff refuse to reveal how they do it. Does this secrecy undermine the scientific validity of the technique?

Scientific science

To what extent is there an overlap between biology and the social sciences? Are the latter 'less scientific'? Consider psychology, sociology, anthropology, and economics.

Knowledge claims

Compare the validity of knowledge claims of two categories of scientific disciplines. For example, you could think about a historical approach (evolution) versus an experimental approach (lab investigation).

Consider these two types of scientific investigation.

1 An archaeological dig searching for evidence of the past.

2 Controlled experiments in a biology lab.

What is nature?

Biology is a natural science, but what is meant by nature? Is it a clockwork machine? Or is it one big Gaia-type living organism? How useful are these metaphors?

Is this a useful image for 'nature'?

Science vocabulary

Does scientific language and vocabulary have a primarily descriptive or interpretive function? Consider the following expressions.

- Natural selection
- Concentration gradient
- Artificial intelligence.

Wiki

Online wikis are filled with user-generated content on a wide range of subjects, including scientific ones. Wikis have been created for scientist to upload their latest laboratory findings. In what ways is this useful to scientists wanting to publish their results? In what ways is this useful to the general public? In what ways does this go against the very nature of peer-reviewed scientific publications, which is the norm today for sharing experimental results? For example, are such wikis just as valid as traditional scientific journals?

What about a wiki or a scientific journal for failed experiments? Why is it that scientists only publish successful investigations and not their failures? If failures were published, couldn't scientists save time by not repeating the same mistakes? Or, could it be that, if another scientist reads what one team thought was a failure but sees it for what it really is, a breakthrough, wouldn't that help science advance?

> "Prediction is very difficult, especially about the future."
>
> Niels Bohr (a Danish physicist who helped us understand how atoms work)
>
> Ellis 1970

Seeing is believing: but what if you cannot see?

There is a story from Asia about a small group of blind men who encounter a tame work elephant, a creature none of them have ever had contact with before.

One blind man touches the elephant's side and says 'It's like a wall'. Another grabs the end of its tail and says 'It's covered in long hairs'. Another feels a leg and says 'Elephants are round and vertical like a pillar'. A fourth holds his ear and says 'It's like a sail'. A fifth holds the animal's trunk and exclaims 'Elephants are like snakes.'

None of the men is wrong but none is completely correct. This story illustrates how easy it is to jump to conclusions before having all the evidence. In science, is it possible to have all the evidence of any particular phenomenon?

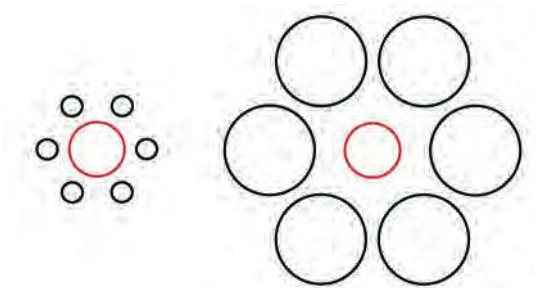
Case study 6 A famous hoax: Piltdown Man

It's always exciting to find a fossil of a new species, especially if it is a hominid. But if you can't find one, should you make one up? In 1912, someone did just that, and called it Piltdown Man. The 'fossil' was made from a human skull and the jaw of an orang-utan. Amazingly, the fake fossil puzzled specialists for more than 40 years before it was finally exposed as a hoax.

This famous hoax demonstrates how important it is to double-check findings. Why did it take so long for the truth to come out? You may find useful online sources if you want to investigate this story.

The Piltdown Man hoax jaw.

Perception



Which red circle is bigger? Judge using your eye first and then use a ruler to check your answer. What does this say about our perceptions and reality?

> "Science may be described as the art of systematic oversimplification."
> Karl Popper
> Popper 1992b

What qualifies as an experiment?

Biology is an experimental science, but what constitutes an 'experiment'? Do you have to have a hypothesis, controlled variables, a laboratory? What if you just have people filling out questionnaires? Is that an experiment? What about digging up fossils?

Theory versus myth

In what ways are theories and myths similar and different? Consider the similarities and differences when comparing and contrasting the two.

Is it based on well-substantiated facts?

Is it passed on from generation to generation?

Can it be modified over time?

Can it be used to predict future events?

Has it been tested repeatedly?

Is it widely accepted as being true?

Is it considered to be a supposition?

Is it considered by many to be false?

> "Irrationally held truths may be more harmful than reasoned errors."
> Thomas Henry Huxley

Biology and values

Do the ends justify the means? Consider the following domains of research in biology. What are the ethical issues?

- Gene therapy
- Vaccine tests
- Experimentation on human volunteers, notably prisoners
- Research involving human embryos.

Moral responsibility

Should scientists be held morally responsible for the applications of their discoveries? Is there any area of scientific knowledge the pursuit of which is morally unacceptable or, on the contrary, morally required?

- Cloning humans
- Eugenics
- Genetic engineering of crops
- Finding a cure for cancer.

> "Nothing in this world is to be feared … only understood."
> Marie Curie

Marie Curie was the first woman to be awarded a Nobel prize and the first person to get two.

Science and religion

To what extent should religion take note of scientific developments? For example, should religious communities keep abreast of scientific discoveries related to Darwin's theory of evolution by natural selection? Some people think that science and religion can coexist; others believe that they are mutually exclusive.

> "Science gets the age of rocks, and religion the rock of ages; science studies how the heavens go, religion how to go to heaven."
>
> Stephen Jay Gould
>
> Gould 2002

Science and technology

Is scientific knowledge valued more for its own sake or for the technology that it makes possible?

Reading your mind

With modern technology tracking everything we do with our computers and smartphones, it can be argued that the kind of privacy our grandparents had no longer exists. Can we at least say that our private and personal thoughts are still safe within our minds and cannot be tracked and monitored?

Functional magnetic resonance imaging technology (fMRI) allows researchers to see which parts of the brain are active when a person is thinking a specific thing or performing a specific task. This has led to the possibility of identifying thoughts or, as some call it, 'mind reading'. For example, researchers have shown a series of images to participants and recorded the patterns that show up on the fMRI scanner for each image. Later, they pick an image at random and show it to the participant while he or she is still in the scanner. A computer can match the current brain scan pattern with one of the patterns observed before and can determine which image the person's brain is perceiving. Experts claim that they can use this technology to see whether someone is lying or to see whether someone recognizes a crime scene that they claim they have never visited. Marketing agencies are interested in seeing how the brain reacts to different advertising campaigns. Some major knowledge issues and knowledge questions arise from this. How can we know if such claims are valid? How do we test them and decide if the scanner and computer are accurate? Should evidence collected in this way be used legally in court as evidence? Could complex thought

Researcher interpreting fMRI scans of the brain.

patterns be identified, such as musical creativity or cruel intentions? Who should decide whether such experimentation and exploration into our private thoughts should be pursued or banned? Would you want a scan done of your thoughts?

Inaccessible worlds

Some scientific fields of exploration have only been possible since suitable technology has been invented, for example genetic engineering has only been possible since technological developments in the 1970s and 1980s. Could there be problems with knowledge that are unknown now because the technology needed to reveal them does not yet exist? Remember that, despite the fact that bacteria are all around us, we were not able to see them until the microscope was invented in the 1600s. Perhaps there are other phenomena that we simply cannot observe because no one has invented an apparatus to detect them yet.

Is there any science that can be pursued without the use of technology?

> "The most important discoveries will provide answers to questions that we do not yet know how to ask …"
>
> John Bahcall (commenting on the Hubble space telescope's capabilities)
> http://en.wikiquote.org/wiki/Science

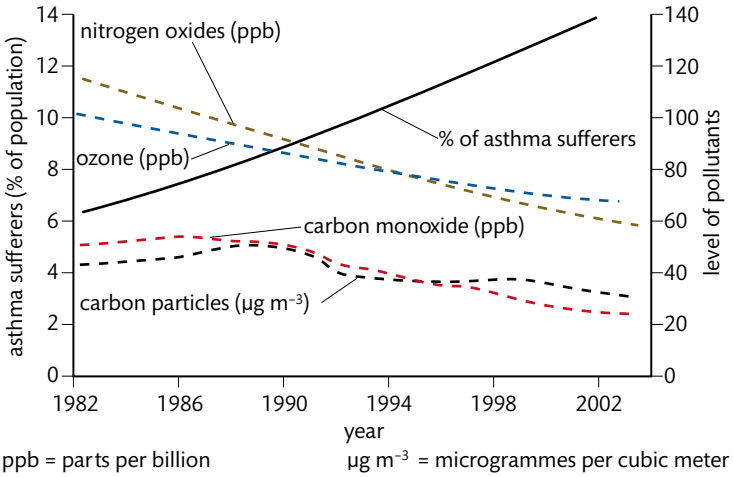
> "My business is to teach my aspirations to conform themselves to fact, not to try and make facts harmonize with my aspirations."
>
> Thomas Henry Huxley

Mathematics, and information and communication technology skills

An important part of being a good scientist and of being a citizen in today's information-rich world is to be number-savvy and tech-savvy. This chapter is divided into two parts: Part 1 Mathematics and statistical analysis, and Part 2 Information and communication technology (ICT) in biology.

In Part 1, we will explore the kinds of mathematical skills needed by a student of biology in order to understand some basic operations and ways of statistically analysing scientific data. Hopefully this section will make you to feel more comfortable with data and give you strategies for understanding graphs and statistical tests that will improve both your internal assessment (IA) work and your exam results.

In Part 2, we will look at how computers, tablets, data-logging devices, and software programs can help us work with numbers and statistics, notably for lab reports.



ppb = parts per billion

μg m^{-3} = microgrammes per cubic meter

Figure 1 Large quantities of data give us superpowers: they allow us to see things other people cannot see. Being able to collect and process data are important skills but also students need to know how to interpret data, including reading graphs, grasping statistics and understanding units and their uncertainties. This graph contains an impressive amount of information in just a few square centimetres – there are 20 years of measurements of five different things. The graph aims to answer the question of whether or not there is a link between asthma and air pollution. Try out your data analysis skills and yours TOK critical thinking skills on this graph.

1 Mathematics and statistical analysis

In the first part of this chapter, you will learn how scientists analyse the evidence they collect when they perform experiments. You will be designing your own experiments, so this information will be very useful to you. You will be learning about:

- means
- error bars
- *t*-tests
- standard deviation
- significant difference
- causation and correlation.

Have your calculator with you to practise calculations for standard deviation and *t*-tests, so that you can use these methods of analysing data when you do your own experiments.

Mean

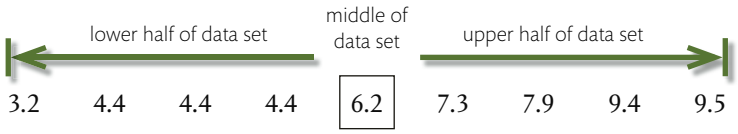
The mean is an average of data points. For example, suppose the height of bean plants grown in sunlight is measured in centimetres (cm) 10 days after planting. The heights for nine of the plants are shown below in cm. The sum of the heights is 56.7 cm. Divide 56.7 by 9 to find the mean (average). The mean is 6.3 cm. The mean shows the central tendency of the data.

3.2 9.5 4.4 6.2 7.9 4.4 9.4 7.3 4.4

In the ICT section of this chapter, you can learn how to use a spreadsheet to calculate the mean and many other values for your data.

Median

Simply put, the median is the number in the middle. It is the number that separates the higher half of the data from the lower half of the data. To find it, the data must first be put in order from the lowest to the highest value. The nine heights of plants have been put in order below.



Because there are nine values, the fifth value separates the lower four from the top four. In cases when there is an even number of data points, take the mean (average) of the two numbers in the middle. For example, if a tenth plant was added to the sample and it was the tallest at 9.7 cm, then the two values in the centre would be 6.2 and 7.3. The mean of those two is 6.75, so the median would be 6.75 cm. But in the example above with nine plants, the median is 6.2 cm.

Mode

The mode is the most frequently occurring measurement. In this case, 4.4 is repeated three times, and no other value is repeated, so the mode is 4.4.

Range

The range is the measure of the spread of data. It is the difference between the largest and the smallest observed values. In our example, the range is 9.5 – 3.2 = 6.3. The range for this data set is 6.3 cm. If one data point was unusually large or unusually small, this very large or small data point would have a big effect on the range. Such very large or very small data points are called outliers. In our sample there is no outlier. If one of the plants died early and had a height of only 0.5 cm, it would be considered to be an outlier. In a lab report, it is acceptable to exclude an outlier from data processing, but it is important to declare it and explain why it was excluded.

Error bars

Error bars are a graphical representation of the variability of data. Error bars can be used to show either the range of data or the standard deviation (SD) on a graph. Standard deviation is explored further on the next page. Notice the error bars representing standard deviation on the bar chart in Figure 2 and the graph in Figure 3.

The value of the standard deviation above the mean is shown extending above the top of each bar of the chart, and the same standard deviation below the mean is shown extending below the top of each bar of the chart. As each bar represents the mean of the data for a particular tree species; the standard deviation for each type of tree will be different, but the value extending above and below a particular bar will be the same. The same is true for the line graph. As each point on the graph represents the mean data for each day, the bars extending above and below the data point are the standard deviations above and below the mean.

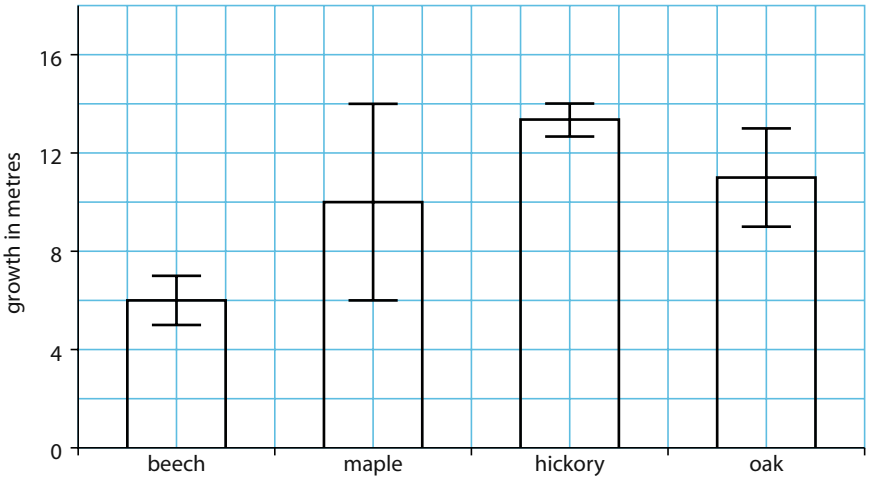


Figure 2 Rate of tree growth on an oak–hickory dune in 2004–05. Values are represented as mean ±1 SD from 25 trees per species.

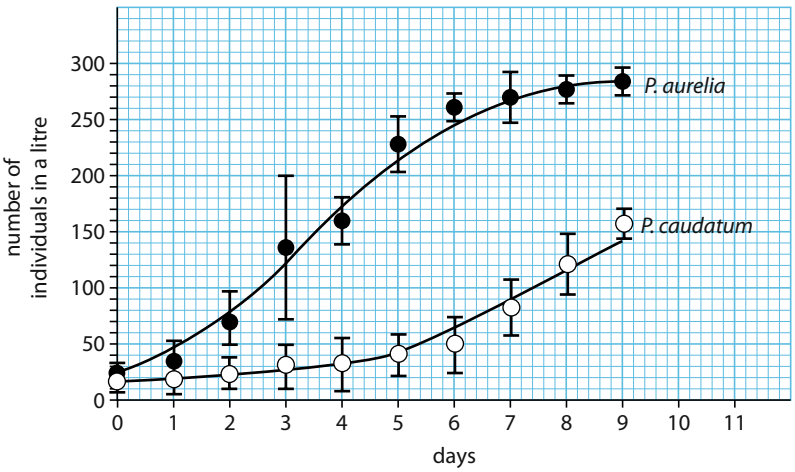


Figure 3 Mean population density ±1 SD of two species of *Paramecium* grown in solution.

Standard deviation

We use standard deviation to summarize the spread of values around the mean, and to compare the means and spread of data between two or more samples. Think of the standard deviation as a way of showing how close your values are to the mean.

In a normal distribution, about 68% of all values lie within ±1 standard deviation (SD) of the mean. This rises to about 95% for ±2 standard deviations from the mean.

To help understand this difficult concept, let's look again at the bean plants. Some bean plants were grown in sunlight, and some were grown in shade. Regarding the bean plants grown in sunlight: suppose our sample is 100 bean plants. Of those 100 plants, you might guess that a few will be very short (maybe the soil they are in is slightly

sandier). A few may be much taller than the rest (possibly the soil they are in holds more water). However, all we can measure is the height of all the bean plants growing in the sunlight. If we then plot a graph of the heights, the graph is likely to be similar to a bell curve (see Figure 4). In this graph, the number of bean plants is plotted on the *y*-axis and the heights, ranging from short to medium to tall, are plotted on the *x*-axis.

Many data sets do not have a distribution that is as perfect as the middle part of Figure 4. Sometimes, the bell-shape is very flat. This indicates that the data are spread out widely from the mean. In some cases, the bell-shape is very tall and narrow. This shows that the data are very close to the mean and not spread out.

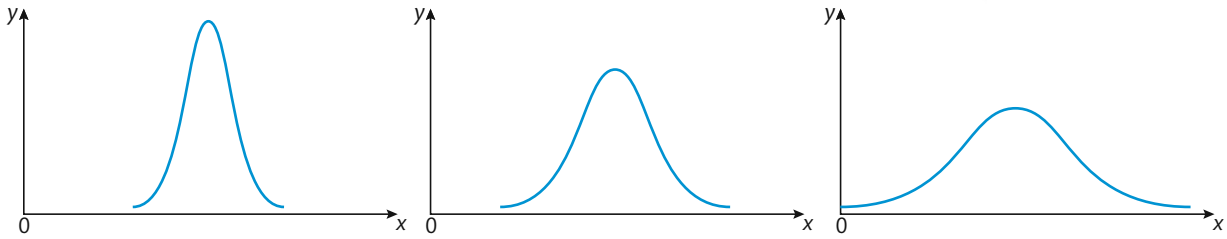


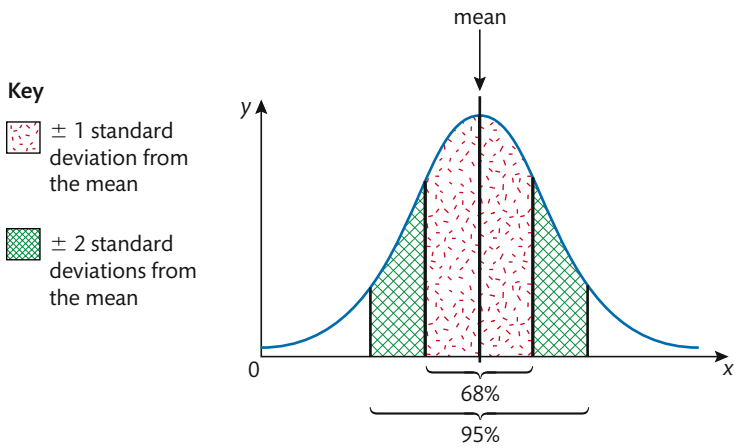
Figure 4 Three different normal distribution curves. The first shows very little spread from the mean, the second shows a moderate amount of spread from the mean, and the third shows a wide distribution of data points from the mean.

The standard deviation shows us how tightly the data points are clustered around the mean. When the data points are clustered together, the standard deviation is small; when they are spread apart, the standard deviation is large. Calculating the standard deviation of a data set is easily done on a calculator with mathematical functions.

For example, if all the students in a year group got 5s and 6s as their marks in a test, the standard deviation would be low, whereas if the results ranged from 2s to 7s, the standard deviation would be higher.

Look at Figure 5. This graph of normal distribution may help you understand what standard deviation really means. The dotted area represents one standard deviation (1 SD) in either direction from the mean. About 68% of the data in this graph are located in the dotted area. Thus we say that, for normally distributed data, 68% of all the values lie within ±1 SD from the mean. Two standard deviations from the mean (the dotted and the cross-hatched areas combined) contain about 95% of the data. If this bell curve was flatter, the standard deviation would have to be larger to account for the 68% or 95% of the data set. Now you can see why standard deviation tells you how widespread your data points are from the mean of the data set.

Figure 5 This graph shows a normal distribution.



How is knowing this useful? For one thing, it tells you how many extreme values are in the data. If there are many extremes, the standard deviation will be large; with few extremes the standard deviation will be small. When processing your data for lab reports, calculating the standard deviation can help you to analyse the data.

Standard deviation is used as an indication of how variable data are. It helps to answer the question 'How far do my data stray from the average?' Data distributed on a normal distribution curve in such a way that most of the data points are near the mean have a low standard deviation (minimal variation), whereas data spread out far from the mean have a high standard deviation (wide variation). Standard deviation can sometimes be used in data processing to help you to decide whether your data are following a clear pattern or whether something is generating unexpected variations.

Comparing the means and spread of data between two or more samples

Remember that in statistics we make inferences about a whole population based on just a sample of the population. Let's continue using our example of bean plants growing in the sunlight and shade to determine how standard deviation is useful for comparing the means and the spread of data between two samples. Table 1 shows the raw data sets at the end of the experiment looking at bean plants grown in sunlight and in shade.

Table 1 Data from a bean plant experiment

Height of 10 bean plants grown in sunlight, in centimetres ±1 cm	Height of 10 bean plants grown in shade, in centimetres ±1 cm
125	131
121	60
154	160
99	212
124	117
143	65
157	155
129	160
140	145
118	95
Total 1310	Total 1300

Bean plants being grown for an experiment.

First, we determine the mean for each sample. As each sample contains 10 plants, we can divide the sum of all the heights by 10 in each case. The resulting means are 131 and 130 cm, respectively.

Of course, that is not the end of the analysis. Can you see that there are large differences between the two sets of data? The heights of the bean plants grown in the shade are much more variable than those of the bean plants grown in the sunlight. The means of each data set are very similar, but the variation is not the same. This suggests that other factors may be influencing growth, in addition to sunlight and shade.

How can we mathematically quantify the variation that we have observed? Fortunately, your calculator should have a function that will do this for you. All you have to do is input the raw data. As practice, find the standard deviation of each raw data set above before you read on.

The standard deviation of the bean plants grown in sunlight is 17.68 cm, while the standard deviation of the bean plants grown in shade is 47.02 cm. Looking at the means alone, it appears that there is little difference between the two sets of bean plants. However, the high standard deviation of the bean plants grown in the shade indicates a very wide spread of data around the mean. The wide variation in this data set makes us question the experimental design. What is causing this wide variation in data? Is it possible that the plants in the shade are also growing

in several different types of soil? This is why it is important to calculate the standard deviation, in addition to the mean, of a data set. If we looked at only the means, we would not recognize the variability of data seen in the shade-grown bean plants.

Significant difference between two data sets using a *t*-test

In order to determine whether or not the difference between two sets of data is a significant difference, *t*-tests are commonly used. The Student's *t*-test (named after a scientist publishing his work under the pseudonym 'Student') compares two sets of data, for example the heights of the bean plants grown in sunlight and the heights of bean plants grown in shade. Look at the top of the table of *t*-values (Table 2) and you will see the probability (*p*) that chance alone could make a difference. If $p = 0.50$, it means the difference could be the result of chance alone 50% of the time.

Statistical significance refers to how probable it is that a relationship is caused by pure chance. If a relationship is statistically significant, it means that there is very little chance that the relationship is caused by chance. We can also use this idea to see whether the differences between two populations are random or not.

For example, a value of $p = 0.50$ (or 50%) is not a significant difference in statistics. It means that there is a 50% probability that the differences are caused by chance alone. However, if you reach $p = 0.05$, the probability that the difference is caused by chance alone is only 5%. This means that there is a 95% likelihood that the difference has been caused by something besides chance. A 95% probability is statistically significant in statistics. Statisticians are rarely completely certain about their findings, but they like to be at least 95% certain of their findings before drawing conclusions.

The formula when comparing two populations that are assumed to have equal variance is as follows:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\left(\frac{(N_1 - 1)s_1^2 + (N_2 - 1)s_2^2}{N_1 + N_2 - 2}\right)\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

Note: you will *not* be asked this formula on exams – it is presented here only as something that might be useful for processing the data collected in your laboratory investigations.

$\bar{X}_1$ = the mean of population 1
$\bar{X}_2$ = the mean of population 2
N = sample size of the population
s = standard deviation

If you plug in the values from the above example with bean plants, you should get $t = 0.06$. You can use a table of critical *t*-values (Table 2) to find out what this number means. To do this, look in the left-hand column of Table 2, headed 'Degrees of freedom', then look across to the given *t*-values. For a two-sample *t*-test like the one we are doing, the degrees of freedom (d.f.) are the sum of the sample sizes of the two groups minus two: 10 + 10 - 2 = 18.

If d.f. = 18, we need to look at the row on the table of *t*-values that corresponds to 18. We see that our calculated value of *t* (0.06) is less than 0.69 on the table, indicating that the probability that the differences between the two populations of plants are due to chance alone is greater than 50%. In other words, we can safely declare that there is no statistically significant difference in the data collected from the bean plants in the sunlight and those from the shade. The differences are most likely due to chance. In order to be able to declare that our two populations showed a level of 95% significance in their differences, we would need a *t* value of 2.10 or more (see d.f. = 18 and $p = 0.05$ (5%) in Table 2). Interpretations of such data processing can be a crucial addition to an effective conclusion on a lab report.

When something is considered to be statistically significant, it means that there is a strong probability that it is *not* caused by chance alone. When something could be caused by chance, we say in statistics that it is not statistically significant.

Table 2 ***t*-values**

		Probability (*p*) that chance alone could produce the difference					
		0.50 (50%)	0.20 (20%)	0.10 (10%)	0.05 (5%)	0.01 (1%)	0.001 (0.1%)
Degrees of freedom	1	1.00	3.08	6.31	12.71	63.66	636.62
	2	0.82	1.89	2.92	4.30	9.93	31.60
	3	0.77	1.64	2.35	3.18	5.84	12.92
	4	0.74	1.53	2.13	2.78	4.60	8.61
	5	0.73	1.48	2.02	2.57	4.03	6.87
	6	0.72	1.44	1.94	2.45	3.71	5.96
	7	0.71	1.42	1.90	2.37	3.50	5.41
	8	0.71	1.40	1.86	2.31	3.37	5.04
	9	0.70	1.38	1.83	2.26	3.25	4.78
	10	0.70	1.37	1.81	2.23	3.17	4.59
Degrees of freedom	11	0.70	1.36	1.80	2.20	3.11	4.44
	12	0.70	1.36	1.78	2.18	3.06	4.32
	13	0.69	1.35	1.77	2.16	3.01	4.22
	14	0.69	1.35	1.76	2.15	2.98	4.14
	15	0.69	1.34	1.75	2.13	2.95	4.07
	16	0.69	1.34	1.75	2.12	2.92	4.02
	17	0.69	1.33	1.74	2.11	2.90	3.97
	18	0.69	1.33	1.73	2.10	2.88	3.92
	19	0.69	1.33	1.73	2.09	2.86	3.88
	20	0.69	1.33	1.73	2.09	2.85	3.85
	21	0.69	1.32	1.72	2.08	2.83	3.82
	22	0.69	1.32	1.72	2.07	2.82	3.79
	24	0.69	1.32	1.71	2.06	2.80	3.75
	26	0.68	1.32	1.71	2.06	2.78	3.71
	28	0.68	1.31	1.70	2.05	2.76	3.67
	30	0.68	1.31	1.70	2.04	2.75	3.65
	35	0.68	1.31	1.69	2.03	2.72	3.59
	40	0.68	1.30	1.68	2.02	2.70	3.55
	45	0.68	1.30	1.68	2.01	2.70	3.52
	50	0.68	1.30	1.68	2.01	2.68	3.50
	60	0.68	1.30	1.67	2.00	2.66	3.46
	70	0.68	1.29	1.67	1.99	2.65	3.44
	80	0.68	1.29	1.66	1.99	2.64	3.42
	90	0.68	1.29	1.66	1.99	2.63	3.40
	100	0.68	1.29	1.66	1.99	2.63	3.39

Worked example

Two groups of barnacles living on a rocky shore were compared. The width of their shells was measured to see whether there was a significant size difference depending on how close they lived to the water. One group lived between 0 and 10 m above the water level. A second group lived between 10 and 20 m above the water level.

The width of the shells was measured in millimetres (mm). Fifteen shells were measured from each group. The mean size of the group living closer to the water indicated that barnacles living closer to the water had larger shells. If the value of t is 2.25, is that a significant difference?

Solution

For one of the steps of the Student's t-test, we need to determine the degrees of freedom. In an example like this one, where the two sample sizes are equal and we can assume the variance in the two samples is the same, the degree of freedom is $2n - 2$. The letter n represents the sample size (the number of measurements made), and in this case $n = 15$. The degrees of freedom in this example is 28 because $(2 \times 15) - 2 = 28$. Looking along the row of Table 2 that shows the degrees of freedom of 28, we see that 2.25 is just above 2.05.

Referring to the top of this column in the table, $p = 0.05$: so the probability that chance alone could produce that result is only 5%.

The confidence level is 95%. We are 95% confident that the difference between the barnacles is statistically significant. In other words, the differences in mean size is very unlikely to be a product of pure chance.

Note: when calculating the t-test value using a spreadsheet program such as Microsoft Excel, be aware that the value obtained is the % chance rather than the value for t. As a result, you do not need to look up the critical values in the table.

Correlation does not mean causation

We make observations all the time about the living world around us. We might notice, for example, that our bean plants wilt when the soil is dry. This is a simple observation. We might carry out an experiment to see whether watering the bean plants prevents wilting. Observing that wilting occurs when the soil is dry is a simple correlation, but the experiment provides us with evidence that the lack of water is the cause of the wilting. Experiments provide a test that shows cause. Observations without an experiment can only show a correlation. Also, in order for these to be evidence of causality, there must be a mechanism to explain why one phenomenon might cause the other. Knowing the properties of osmosis and turgidity in plant cells would explain the causality associated with the correlation, thus giving it great scientific plausability.

A cormorant.

Cormorants

When using a mathematical correlation test, the value of the correlation coefficient, r, is a measure of the degree of linear relationship or linear dependence between two variables. This can also be called the Pearson correlation coefficient. The value of r can vary from +1 (completely positive correlation) to 0 (no correlation) to –1 (completely negative correlation). For example, we can measure the size of breeding cormorant birds to see whether there is a correlation between the sizes of males and females that breed together.

Table 3 Cormorant size data

Pair number	Size of female cormorants, cm	Size of male cormorants, cm
1	43.4	41.9
2	47.0	44.2
3	50.0	43.9
4	41.1	42.7
5	54.1	49.5
6	49.8	46.5
$r = 0.88$		

Correlation does not necessarily mean causality. Just because two things show a relationship and have a strong *r*-value, does not mean one causes the other.

The *r*-value of 0.88 shows a positive correlation between the sizes of the two sexes: large females mate with large males. However, correlation is not cause. To find the cause of this observed correlation requires experimental evidence. There may be a high correlation, but only carefully designed experiments can separate causation from correlation. Causality requires that the mechanism of exactly how X causes Y needs to be demonstrated. For example, the mathematics here does not explain whether it is the males choosing the females or the females choosing the males. Correlation says nothing about the direction of the influence.

Graphs

Scientists use graphs extensively because they are useful tools for presenting data and seeing relationships that might otherwise remain hidden. Graphs are instrumental in analysing data, and if you know how to make accurate and appropriate graphs your conclusion and evaluation will be greatly enhanced.

The most common forms of graphs you are expected to be able to use are:

- bar charts
- histograms
- line graphs
- scatter plots.

Occasionally, you may also need to use pie charts or box and whisker diagrams, but here we will focus on the four listed above.

Bar charts

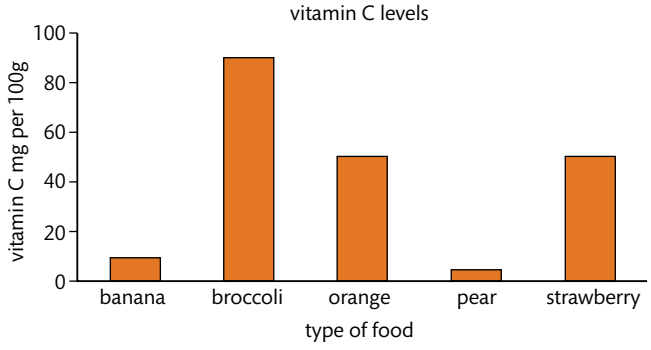


Figure 6 A bar chart showing vitamin C levels in different types of food.

Bar charts use rectangles to show the amount of data in a certain number of categories. The height of each rectangle corresponds to a quantitative value. The *y*-axis is quantitative, but the *x*-axis shows categories rather than incremental numerical values. The order of these categories could be changed and it would not make a difference. Empty spaces separate the rectangles along the *x*-axis. For example, Figure 6 is a bar chart showing the amount of vitamin C in various foods.

In a graph of this type, it is okay to rearrange the bars anyway you want. In Figure 6, the data are presented alphabetically, but there is no reason why you couldn't order the bars from the greatest to the smallest numerical values.

Histograms

Histograms have some similarities with bar charts, except that the *x*-axis has a quantitative scale marking off intervals of continuous data. In addition, the widths of the rectangles that make up the histogram represent specific incremental quantitative values. The histogram in Figure 7 shows the amount of time that 42 individuals of a particular species of animal spent drinking at a river.

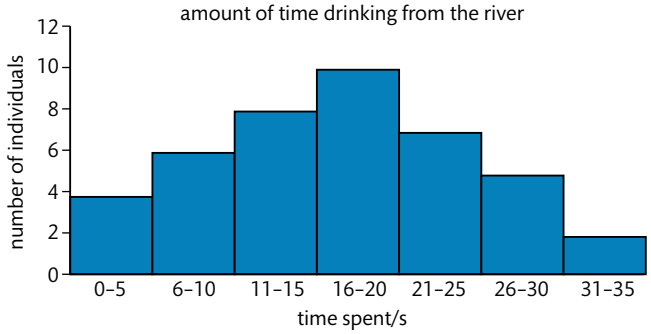


Figure 7 A histogram of the time spent by individuals of a species drinking from a river. Notice the lack of space between the categories, and the fact that the categories on the *x*-axis represent continuous incremental numerical values.

Histograms have no spaces between the rectangles because the data are continuous. This was not the case in the bar chart that we looked at in Figure 6. In Figure 7, we cannot rearrange the rectangles of the histogram so that the highest values are on the left and the lowest values are on the right, as we could have done for the bar chart. Histograms must follow the scale shown on the *x*-axis. If an animal drank for 24 seconds, the data must go in the range 21–25. These ranges can also be called bins, and you can think of a histogram as a series of bins that you fill up with the appropriate data as the data are sorted.

Line graphs

A line graph plots single points over regular increments such that each *x*-value has only one corresponding *y*-value. The dots are then joined with straight lines. The example in Figure 8 shows a newborn baby's body mass between the time of its birth and the age of 18 months.

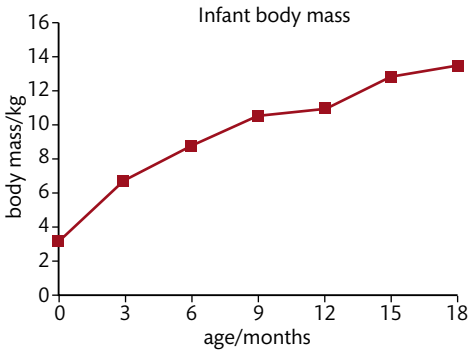


Figure 8 A line graph of infant body mass. Notice that the data points are connected by straight lines rather than using a trend line or line of best fit.

In line graphs, the *x*-axis is usually the independent variable, in which case the *y*-axis is the dependent variable. There is clearly a correlation in this graph: as age increases, body mass increases. There is a positive correlation. But remember, that does not mean there is causality. Ageing is not the mechanism that causes an increase in the child's body mass; on the contrary, good nutrition, genes, and growth hormones are more likely candidates for causing the increase. Line graphs can sometimes show discrepancies in the data. For example, a doctor might wonder why a child did not grow as fast between the ages of 9 and 12 months compared with the rest of the graph. Perhaps the child did not have access to proper nutrition during that interval.

Scatter plots

A scatter plot is used when two variables are involved, and they are plotted as *y* against *x* using Cartesian coordinates. Such graphs work well for situations where one *x*-value may have multiple *y*-values. As with line graphs, scatter plots are useful for trying to see a correlation. Figure 9 shows a scatter plot for the numbers of pairs of grey partridges (a type of bird) plotted against the number of sightings of birds of prey per square kilometre (km^{-2}).

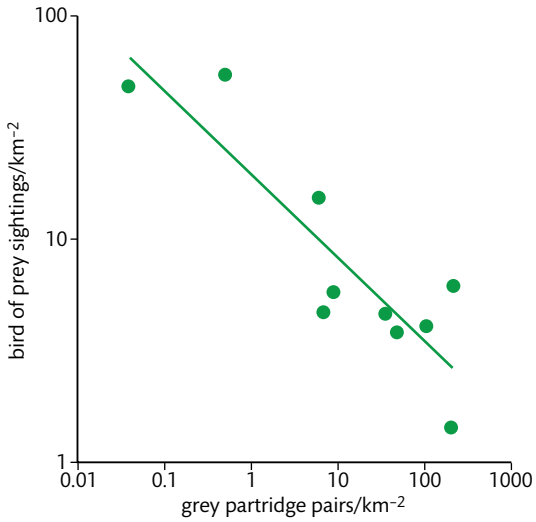


Figure 9 A scatter plot of grey partridge pairs against bird of prey sightings, with logarithmic scales on the *x*- and *y*-axes. M. Watson et al. 2007

Notice how the dots are a bit irregular: this scattering is where this type of graph gets its name from. Notice also how the data points are *not* connected by a line. Rather, a line of best fit or a trend line has been placed over the graph showing an overall trend in the data. Such lines or curves do not need to pass through each data point, as we saw in the line graph. The trend line in Figure 9 shows that there is a negative correlation. A negative correlation means that as one variable increases the other decreases.

Do you notice anything peculiar about the axes? They are shown using a logarithmic scale, which means that each increment is 10 times the size of the one before. This is relatively exceptional: most scatter plots have standard incremental scales on the *x*- and *y*-axes, the way the line graph does in Figure 8. Logarithmic scales are useful when you are trying to show distributions of data points that would not show up if they were put on a normally incremental scale. In this case, it is likely that the authors of the report in which this graph appeared wanted to show the correlation between the sightings of birds of prey and the number of couples of partridges. As we know that there is a logical mechanism for causality (birds of prey kill and eat partridges), it is not impossible to suspect that there is a causal relationship here. But this graph alone cannot prove that birds of prey cause the reduction in numbers of partridges.

Regression models and coefficient of correlation

When scientists measure something, often they are looking to see whether they can demonstrate that the phenomenon is following a law of nature. Sometimes laws of nature follow patterns that can be expressed in mathematical equations. For example, when measuring the light that a leaf might use for photosynthesis, a scientist knows that the intensity of the light varies according to an equation relating intensity with the

distance to the light. You know from personal experience that holding a torch close to your eyes can be blinding, whereas seeing the same torch from far away does not hurt your eyes. In Figure 10, the graph on the left illustrates the 'pure' mathematical law about light intensity and distance from the light source. On the right is the same graph superimposed with measurements taken in a lab. Because of any number of things, including limitations in the equipment and human error, the lab measurements do not fit the mathematical model perfectly.

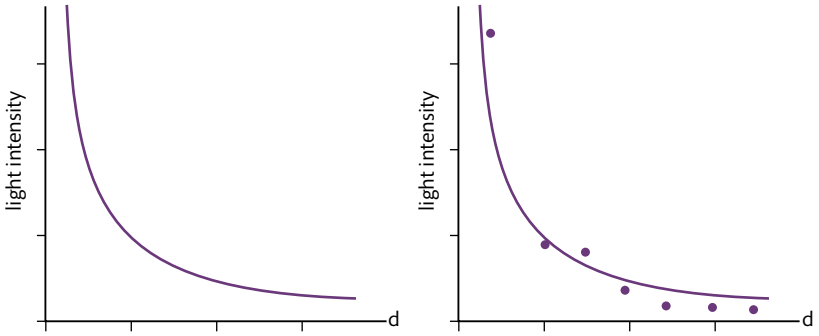
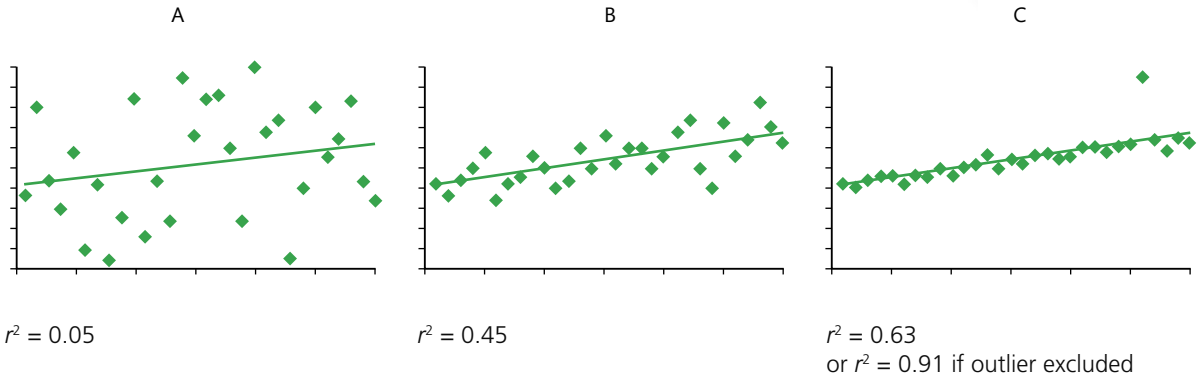


Figure 10 A model of what the data should show (on the left) and the actual collected data (dots on the right), of which only one point is actually where it was expected to be. The d on the *x*-axis of the graphs represents the distance from the light source.

Now imagine the opposite. A scientist takes some measurements and wonders if there is some kind of mathematical equation that could act as a model of her data. She makes a scatter plot and then sees if there is a trend line that fits her data reasonably well. She might start with a straight line because that is the simplest relationship between two variables. This is called a simple linear regression model. But if that does not fit her data well, she could try other regression models that are not straight lines. Fortunately, statistical functions in her calculator or spreadsheet program on her computer can do this for her in an automated fashion.

How can we know if the trend line's regression model is the best one for the data we collected? The squared correlation coefficient, r^2, also called the coefficient of determination, is used to see how well a regression model matches the data collected. A value of $r^2 = 0$ means the regression model does not fit the data at all, whereas a value of $r^2 = 1$ means a perfect fit. Note that r^2 cannot be a negative number. Here are some examples showing the r^2 values calculated by Microsoft Excel for three data sets and their trend lines.

Figure 11 Three examples of data that have been modelled with a linear regression. The r^2-value is then calculated to see how closely the linear regression model matches the data.



Notice what happens to the r^2-value as the variability of the data points is reduced from A to B to C. This reveals that the regression model shown by the trend line matches the observed data better and better. Graph A's regression line suggests that there is very

- Trend lines are useful for seeing whether there is an overall pattern or tendency in the data points.
- The r^2-value, the coefficient of determination, is useful for seeing if the trend line matches the data points closely or not. It indicates how good the model is. The closer it is to 1, the better the model. Values close to 1 reveal that there is a strong correlation between the *x*- and *y*-values.
- If the regression model fits the data well, it can be used to predict values that were not measured.

little evidence of an agreement between the regression model and the data, whereas B and C show a stronger fit. Notice what happens in graph C: there is clearly an outlier at the top right. Fortunately, the investigator identified it as being a result of an error during the lab. It can safely be ignored, and therefore the value of 0.91 can be used for analysis purposes. Students are encouraged to used trend lines and r^2-values in their data processing, in order to analyse the data they have collected better.

In addition to simply seeing whether the data points follow a predictable pattern, a regression model can be used to predict values that were not measured. Knowing the equation of the line or the curve allows a researcher to plug in hypothetical values and get a prediction from the model. For example, changes in the human population in the coming decades can be predicted based on a regression model of current trends in the population. When using a regression model for prediction purposes, the r^2-value can help give a sense of how reliable the prediction will be. For example, predicting an outcome using graph A above would be extremely unreliable. However, using C's regression model would be more likely to give reliable results.

Before and after: by how much did this change?

Sometimes we need to analyse how something has changed over time, or we need to see whether there is a difference between what we expected and what we got.

The simplest way to see a difference is to subtract the 'after' value, V_2, from the 'before' value, V_1. However, it is often practical to calculate a percentage change:

$$\text{percentage change} = \left(\frac{V_2 - V_1}{V_1}\right) \times 100$$

Expected versus observed values: first application of the chi-squared test for goodness of fit

As we saw in Figure 10, we do not always get what we expect with our results. The difference between the expected values and the observed values may simply be caused by chance or, on the contrary, may be because an unexpected phenomenon is having an effect on the data. How can we know? One way to answer this question is to carry out a statistical test called the chi-squared (χ^2) test, which calculates how close our observed results are to the expected values. Chi is the Greek letter χ and is pronounced like the word 'sky' without the s at the beginning.

The first way we will use the χ^2 test is to compare our observed results with what we can theoretically calculate the results should be (the 'expected' results). As you saw in Chapter 10, to use this statistical test it is important to note down carefully all the observed results (O) and the expected results (E). In the case of genetics exercises, the expected results would be the proportions of phenotypes as determined by a Punnett grid, such as 25%/50%/25% or 25%/75%, although it is important to use the actual numbers of offspring rather than percentages or ratios. Setting up a table to help keep track of the numbers is helpful.

Table 4 Charting observed and expected results

	Possible outcome 1	Possible outcome 2	Sum
Observed numbers in each category of possible outcomes (O)			
Expected numbers in each category of possible outcomes (E)			
Difference (O – E)			
Difference squared $(O - E)^2$			
$\frac{(O - E)^2}{E}$			$\chi^2 =$

The third and fourth lines of this table are intermediate steps to see the difference between the observed and the expected values as well as their squared values.

The bottom right cell of the table is what we want: it shows the sum of the last row's values and this is the χ^2 value we are interested in. In effect, the contents of this table can be summarized in the generalized formula for calculating χ^2, which is:

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

where χ = Greek letter chi, O = observed values (the results of the experiment), E = expected values (calculated theoretically), Σ = sum of all the calculations for each type of outcome.

Interpreting the χ^2 value calculated

Once we know the χ^2 value, we need to know what it means. For this there are some concepts that need to be clarified. First of all, there is the concept of the null hypothesis (H_0). The H_0 in an experiment of this type is what we would expect: this is usually determined by mathematical calculations. The χ^2 value will help us to determine whether the null hypothesis can be rejected. Accepting the null hypothesis is a way of saying 'Yes, there is a high probability that any deviation from the expected values can be attributed to chance'.

Another two important concepts to understand are the idea of degrees of freedom (d.f.) and how the idea of probability (*p*) is used. When using the χ^2 test to determine whether there is a difference between the expected and the observed values, the degrees of freedom is determined by taking the number of categories into which the data fall and subtracting 1 from that number. In Table 4, there are two categories into which the data fall (possible outcomes 1 and 2, so there is 2 – 1 = 1 degree of freedom). This number allows us to know where to look in the table of critical values for χ^2 (Table 5). Notice in Table 5 that, in addition to the degrees of freedom, there are probability values for *p*. It is a convention in biology to look for probabilities of 5%, or 0.05.

Do not confuse Tables 2 and 5. Although they both refer to probability and hypothesis testing, the former is for the *t*-test and this one is for the chi-squared test.

Table 5 Critical values for χ^2

		Probability values (p)				
		0.1	0.05	0.025	0.01	0.005
Degrees of freedom (d.f.)	1	2.706	3.841	5.024	6.635	7.879
	2	4.605	5.991	7.378	9.21	10.597
	3	6.251	7.815	9.348	11.345	12.838
	4	7.779	9.488	11.143	13.277	14.86
	5	9.236	11.07	12.833	15.086	16.75
	6	10.645	12.592	14.449	16.812	18.548
	7	12.017	14.067	16.013	18.475	20.278
	8	13.362	15.507	17.535	20.09	21.955
	9	14.684	16.919	19.023	21.666	23.589
	10	15.987	18.307	20.483	23.209	25.188

Look at Table 5 and find the critical value that is of interest to us: it is the one that lines up with a probability value of 0.05 and a degree of freedom of 1. You should get 3.841. This means that any value we calculate for χ^2 that is greater than 3.841 tells us to reject the null hypothesis.

Here is a summary of the steps.

1. Determine the expected values (although we sometimes like to use percentages or proportions in science, the χ^2 test requires numbers here: do not use percentages or ratios).
2. Note down the observed values and decide what the null hypothesis will be.
3. Calculate the value for χ^2 by determining the differences between the values $(O - E)$, then square them, $(O - E)^2$, and finally add them all up.
4. Determine the degrees of freedom (d.f.) by taking the total number of classes into which the data fall and subtracting 1.
5. Look at the table of critical values of χ^2 and use the d.f. and *p*-value (conventionally we use 0.05 for *p*) to determine which critical value ($\chi^2_{critical}$) to compare the calculated value of χ^2 ($\chi^2_{calculated}$) to.
6. Compare $\chi^2_{critical}$ to $\chi^2_{calculated}$ and decide if the null hypothesis can be rejected using these rules:

$\chi^2_{calculated} < \chi^2_{critical}$ — do not reject null hypothesis, any deviations from the expected values are probably the result of chance alone

$\chi^2_{calculated} > \chi^2_{critical}$ — reject null hypothesis, deviations from the expected values are *not* the result of chance alone

If the calculated value for χ^2 is less than the critical value, the null hypothesis cannot be rejected, whereas if the calculated value for χ^2 is greater than the critical value, the null hypothesis can be rejected.

Independent or correlated: second application of the chi-squared test as a test for independence

As seen in Chapter 4, sometimes we need to know whether it is likely that two phenomena are independent from each other or associated with each other. This next application of the χ^2 test will also compare expected and observed values, but this time the expected frequencies are not given in advance. The use of a contingency table like the one below is necessary to determine them. Table 6 shows the data relevant to the quadrat experiment described in Chapter 4, in which students wanted to see whether the distribution of ferns was random or whether they were found more commonly in sunny or shady areas.

Table 6 Quadrat data

Observed:		Area sampled		
		Sunlight	Shade	
Presence of ferns	Present	7	14	21
	Absent	13	6	19
		20	20	40

The cells in pink show the two columns and two rows of observed data; the yellow cells show the marginal totals for the two rows; and the blue cells show the marginal totals for the two columns. The number 40 represents the whole sample size of 40 quadrats (20 from the sunlit areas, and 20 from the shaded areas).

Determining the expected values

Unlike the previous use of the χ^2 test, we have no mathematical model to predict the theoretical 'expected' values. For that, we construct a new table by removing the observed values.

Table 7 Determining the expected values, step 1

		Area sampled		
		Sunlight	Shade	
Presence of ferns	Present			21
	Absent			19
		20	20	40

Now, to fill in the table with expected values, we multiply the marginal total of each row by the marginal total of each column.

Table 8 Determining the expected values, step 2

Expected:		Area sampled		
		Sunlight	Shade	
Presence of ferns	Present	(20 × 21) ÷ 40 = 10.5	(20 × 21) ÷ 40 = 10.5	21
	Absent	(20 × 19) ÷ 40 = 9.5	(20 × 19) ÷ 40 = 9.5	19
		20	20	40

There are some conditions that need to be met when using the χ^2 test.

- **This kind of statistical test works with data that you can put into categories and you want to find out whether the frequency that the results fall into a particular category is the result of chance alone.**
- **Make sure the categories into which the data can fall are exhaustive and mutually exclusive, such as yes/no, or red flower/white flower/pink flower. As with flipping a coin, heads/tails, all the data collected must fall into one or the other of the categories.**
- **Make sure the data sample is sufficiently large: with fewer than five data points in any one category, the result will not be very reliable.**

The null hypothesis is usually the opposite of the investigator's hypothesis. For example, if a doctor wanted to study the effects of a drug on her patients, she might have the hypothesis 'This drug has a positive influence on my patients' health. Compared with the control group not taking the drug, the experimental group will declare more often that they feel better.' In such a scenario, the null hypothesis would be: 'This drug has no influence on my patients' health. There is no difference between the control group and the experimental group: I can be confident that any observed differences will be due to chance alone.'

This is why researchers are happy and satisfied when they can reject the null hypothesis. They are glad to see that they can rule out the idea that the results are only caused by chance. But be careful: just because the null hypothesis can be rejected, it does not mean that the investigator's hypothesis has been validated.

Degrees of freedom

To determine the degrees of freedom, take the number of rows (r) minus one and multiply that by the number of columns (c) minus one. In this case, there are two columns (sunlight and shade) and two rows (ferns present and ferns absent), so the formula is:

$$\text{d.f.} = (r-1)(c-1)$$

$$\text{d.f.} = (2-1)(2-1) = 1$$

Calculate the chi-squared value

For most tables of contingency, the normal formula for χ^2 can be used:

$$\chi^2 = \Sigma\frac{(O-E)^2}{E}$$

Your calculator or spreadsheet program most likely has formulas to calculate this value quickly, but using tables like Table 4 can allow you to walk through the calculation step by step. You should get 4.91 as the critical value of t.

Test for independence: interpreting the chi-squared value

Now we need to look at the critical values table (Table 5). As we have 1 degree of freedom and we always look at $p = 0.05$, the critical value comes out as 3.841. As our calculated value (4.91) is higher than this critical value, we can safely reject the null hypothesis. What does this mean? It means that if the null hypothesis were true and the distribution of ferns was solely the result of chance, there would be less than a 5% chance of getting the results we observed. The fact that our calculated χ^2 value is high means that the relationship is statistically significant. It also means that the distribution of ferns and the presence of sunlight are not totally independent from each other. We can reject the idea that they are independent.

2 Information and communication technology in biology

In the second part of this chapter, we will look at how digital technology can be applied to biology. Thanks to advances in desktop computers, laptops, tablets, and smartphones, many tools that would have only been available to highly specialized labs a few decades ago are now available to everyone. In the 1980s, for example, three-dimension (3-D) animation was cutting-edge technology requiring a roomful of computer processors. Today, teenagers can sketch objects in 3-D on their smartphones. We will look at the following aspects of Information and communication technology (ICT) that apply to biology:

- models
- simulations
- databases
- questionnaires and surveys
- data-analysis exercises
- fieldwork and data logging
- ICT skills as applied to lab reports.

Models

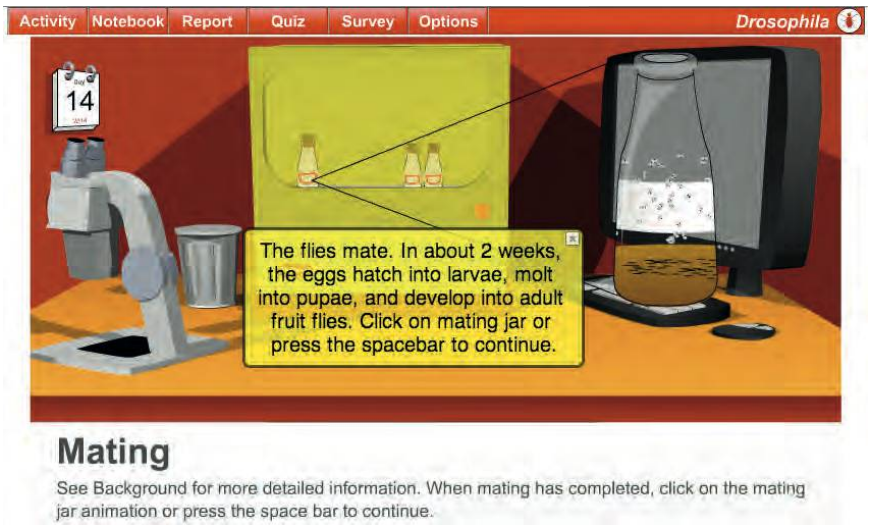
A model is a simplified representation of an object or a phenomenon that can be used to better explain or understand it. Physical models, such as a plastic model of a heart, might help a student to see how the valves work to keep the blood flowing in one direction between the chambers. Computer models, such as a 3-D animation of a beating heart or abstract models showing a flowchart of a process such as DNA replication, can help the learner grasp complex concepts by providing simplified visualizations. Computational models, such as climate models, might be used to help simulate Earth's true climate on a computer.

A computer model can be used to study 3-D objects. Once an object such as this skull is represented as a 3-D model, the data can be shared with other labs and be studied by multiple experts all over the world simultaneously.

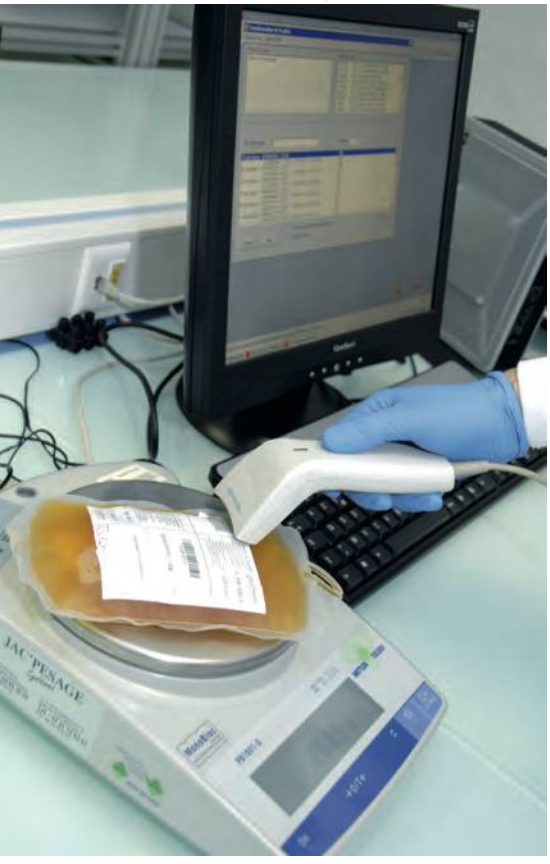
Simulations

Models can be applied in simulations in order to represent a process or system. Because variables can be manipulated within them, simulations are often used to predict an outcome or to find out what the optimum parameters are for a system. For example, computer simulations use climate models to predict what will happen to Earth's climate if carbon dioxide levels increase. In the lab, some experiments are too

dangerous, too time-consuming, or too costly to carry out; computer simulations of those experiments can be performed on a computer safely and in a time-saving fashion. Experiments mating fruit flies, for example, would take many weeks to do, or experiments on the effects of introducing predators into an ecosystem would take months or years, and not be very realistic for an IB student to undertake. However, computer simulations can allow a student to collect data and perform experiments virtually on screen. See the hotlinks section at the end of this chapter for examples of online simulations.



A simulation for mating fruit flies to see what kinds of genetic combinations are possible. Notice that this simulation uses models of a microscope, an incubator, glassware, and a lab bench to simulate this experiment with virtual flies that follow a genetic model. sciencecourseware.org



Entering data about blood plasma into a database using a bar code reader.

Databases

Students are often encouraged to compare the values they get in the lab investigations they carry out with values that scientists in other labs have obtained. In other instances, students do not have access to the lab equipment necessary to do certain experiments, such as finding gene sequences or measuring carbon dioxide concentrations over many decades. In either case, databases are available online for a variety of types of data, and students should take advantage of these resources. Hotlinks to some useful databases can be found at the end of the chapter.

Gathering your own statistics

Questionnaires and surveys can sometimes come in handy when students are looking for large quantities of data to analyse. Writing a good survey or questionnaire is an art as well as a science. As with many projects, once you have an idea for your research question, it is best to start with the end in mind and then work backwards.

1. Picture the kinds of graphs that you would want to see on your final data processing that would lead you to an interesting conclusion.
2. Then think of what kinds of data need to be collected in order to produce such graphs. For example, suppose you want to

find out whether the use of flashcards helps students perform better when doing biology multiple-choice questions. You would need to decide whether you want to ask how many flashcards students have made or how much time they spend reviewing them, or both.

3 Because you are trying to show the influence of X on Y, you would probably want to do some kind of scatter plot graph with a trend line to see whether there is a positive or negative correlation. Perhaps you could do some data processing to find out whether the number of cards and/or the amount of time they are used is independent of the students' test scores or not. You could calculate whether there was a statistically significant difference between one group and another in terms of test performance.

4 To see if there is an influence, you would need to obtain the test scores from the participants in your study. This raises some ethical questions because certain students might not want to give you that information. Every time you do a questionnaire, you must tell the students what information is being collected, why it is being collected, and what will be done with the information. They have the right to know, for example, if your data is going to be shared with other people. If your intent is to collect anonymous data, you can reassure your participants that their names will not appear anywhere in the data. Also, you should give the participants the opportunity to leave certain questions blank.

5 Use these helpful hints about setting up questionnaires and surveys.

   (a) Even if your questionnaire is anonymous, be sure to collect some demographic information, such as female/male, age, year group, etc. Put such questions at the end of your questionnaire or survey rather than at the beginning. This information might prove useful later because you might see some unexpected trends in the data, such as which age groups use flashcards the most. Some of the best discoveries are the unexpected ones.

   (b) So that the data are easier to use in a spreadsheet, use tick boxes or multiple-choice questions whenever possible. Avoid open-ended questions where participants write their own answers. For example, in an open-ended question about gender, some participants may write 'male' and others may write 'M'. A computer would see that as two different answers, even though we know they both mean male.

   (c) Be sure that your categories do not overlap. For example, if you are asking students to tick their age group, do not put '13 to 15' as one category and '15 to 17' as another category because students who are 15 will not know which one to tick.

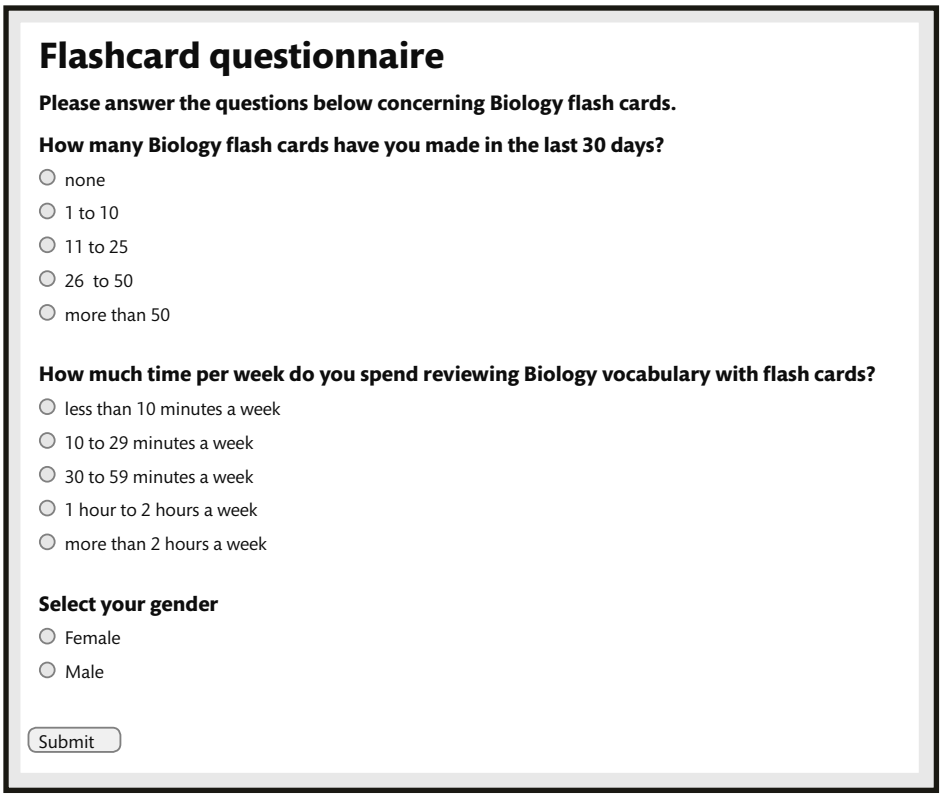
   (d) Before you send out your questionnaire to all the participants, try it out on a few classmates and teachers. Often they can spot errors that you did not see or they might have suggestions for clarifying certain points.

Although it is possible to print out and photocopy sheets to collect data, it is very time-consuming to type in all the answers into a spreadsheet when you want to do your data processing. It is much quicker to set up an online questionnaire so that as soon as participants click 'submit' the answers are added to a spreadsheet. The hotlinks at the end of this chapter have some suggestions for online questionnaire and survey websites.

In addition to following the IB's guide concerning ethical questions applied to experimentation in the lab, students interested in writing questionnaires might want

Google Forms is a no-cost solution for making an online survey. Some online services for surveys limit the number of respondents or charge a fee if you get more than 100 respondents, but this is not the case for Google Forms.

to have a look at the diploma programme guide for psychology. In it, there are clear guidelines about what the IB considers to be acceptable practice when using human subjects for an investigation.



Flashcard questionnaire

Please answer the questions below concerning Biology flash cards.

How many Biology flash cards have you made in the last 30 days?

- none
- 1 to 10
- 11 to 25
- 26 to 50
- more than 50

How much time per week do you spend reviewing Biology vocabulary with flash cards?

- less than 10 minutes a week
- 10 to 29 minutes a week
- 30 to 59 minutes a week
- 1 hour to 2 hours a week
- more than 2 hours a week

Select your gender

- Female
- Male

Submit

Figure 12 Example of three questions from an anonymous online questionnaire using multiple-choice questions from Google Forms. Other questions might gather data about students' results on their last biology test or whether they are taking HL or SL Biology.

Data-analysis exercises

Both for your internal assessment work and in data-based sections of exams, you will be required to interpret sets of data presented either as tables or as graphs. Being able to extract scientific information from data is a key skill in biology.

The first thing to look for on a table or a graph is a title. When titles are not available, often the text before or after the tables and graphs will reveal some key information about what they are showing. The next clues to look for in order to interpret the data correctly are labels and units in the headings of tables, or labels and units on the axes of graphs. In both cases, the labels are often the dependent and independent variables of the investigation that generated the data. Knowing these will help you reach conclusions about the investigation. The units might be familiar to you, such as grams, millilitres, or °C, but sometimes they are units you have never heard of. In such cases, do not panic, just be sure to include those unfamiliar units in your answers and in your analysis. The same goes for arbitrary units, which are sometimes used to avoid employing confusing units.

Next, look at the scales on the axes of graphs. Do they show regular intervals (10, 20, 30, 40) or is there an atypical scale, such as a logarithmic scale (1, 10, 100, 1000)? If two graphs are being compared, do they use the same scales and the same maximum and minimum values? If not, be careful with how you compare the two because they may look the same but in fact be very different.

Worked example

Analyse the graph below showing the sizes of wings of fruit flies in Europe, North America, and South America. Note that the original species of *Drosophila subobscura* lived in Europe and was introduced to the Americas in recent decades. What scientific information can be concluded from the graph? For example, can any predictions be made?

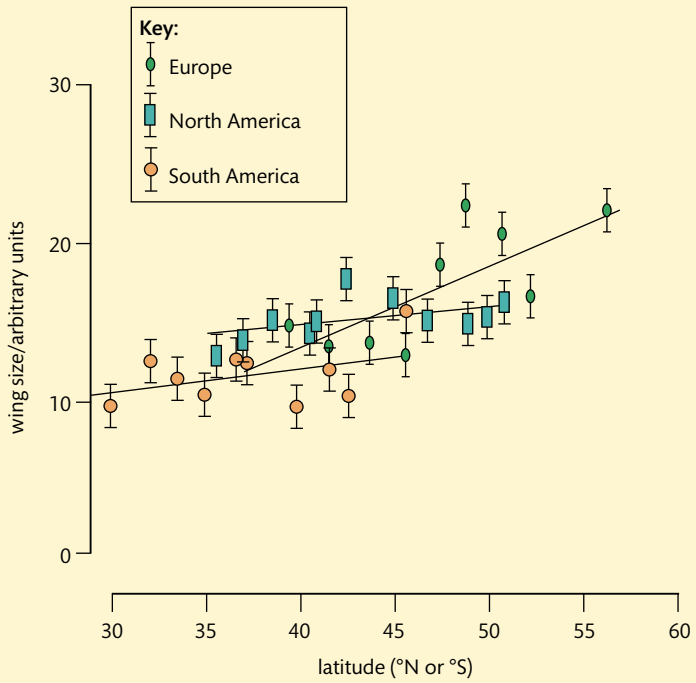


Figure 13 Graph showing fruit fly *Drosophila subobscura* wing sizes in different parts of the world.
Gilchrist et al. 2004

Solution

Reading the graph: look at the axes, showing latitude for the *x*-axis and wing size for the *y*-axis. Latitude is a measurement of how many degrees away from the equator something is: low numbers are closer to the equator, high numbers are further away from the equator. Next, look at the key: there are three different shapes and colours to analyse, depending on where the flies were observed. Associated with each group is a trend line. In addition, each data point has vertical error bars.

Analysis of the graph: all three trend lines increase as the latitude gets further from the equator, but the one from Europe has the greatest slope. If we look at the centre of each trend line, it appears that the South American population has the smallest wing size, the North American population has an intermediate wing size, and the European population has the biggest wing size. The European population has the widest range of wing sizes.

Conclusion: There is a relationship between latitude and wing size: they are positively correlated. We can predict that if the introduced populations of *D. subobscura* in the Americas were to spread to latitudes that are further away from the equator than the ones show in the graph, they should show an increase in wing size.

Throughout this book, there are examples of past paper questions, and some of them have graphs or tables of numbers that need to be interpreted and analysed. Be sure to practise analysing them because that is what you will be asked to do in exams.

Fieldwork and data logging

Biology investigations carried out in labs allow students to have a certain amount of control over the variables that they are manipulating. Fieldwork, however, does not offer such possibilities. Studying a forest, stream, grassland, or marine environment poses some unique challenges. Abiotic factors, such as temperature, air humidity, and light, can vary considerably, and could have an influence on what is being studied. For example, setting up pitfall traps is a wonderful way to collect invertebrates in a forest or grassland, but adverse weather conditions might greatly affect how active invertebrates are. Because they cannot be controlled, abiotic factors should be monitored and data should be collected to make sure that they do not have an adverse effect on the results.

A student using a hand-held data-logging device to measure the pH of a sample of water in a river.

Collecting large quantities of data can sometimes be tedious and prone to errors if done by hand. Instead of using a thermometer and writing down the temperatures, students can use temperature probes connected to data-logging devices that can automatically record temperatures at particular intervals. Such devices can be equipped with probes for:

- temperature
- light intensity
- relative humidity
- flow rate (to see how fast water is flowing)
- dissolved oxygen.

Data loggers can also have an integral global positioning system (GPS) (to record the exact location of each measurement).

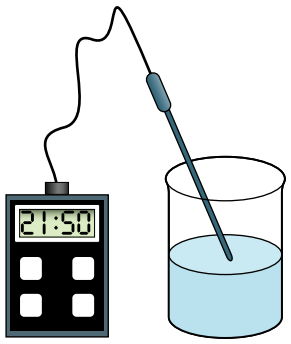


Figure 14 A hand-held data-logging device with a probe measuring the temperature of a solution in a beaker.

Once the probes are plugged in, the data-logging device can be used in various modes depending on how the data are to be collected. Here are some examples:

- real-time (useful for monitoring measurements without recording them; in this mode, the device is used as a simple meter)
- events with entry (useful for measuring a certain factor every metre, or when another event happens; data will only be recorded when you tell the device to do so)
- time-based (useful for measuring something constantly over a fixed amount of time; in this mode, parameters can be adjusted to measure every minute, hour, second or fraction of a second).

Although the data can be transferred to a computer, many of these devices allow you to graph and analyse the data directly on screen. This is especially useful when doing fieldwork without a readily available computer.

ICT skills applied to your lab reports

To produce top-quality lab reports, it is expected that students have access to the following types of programs.

- Word processing software: programs such as Microsoft Word will help you with text, tables, footnotes, and chemical as well as mathematical formulas.
- Spreadsheet software: programs such as Microsoft Excel will help you with data processing to perform calculations such as averages, standard deviation, chi-squared tests, and more.
- Graphing software: in addition to its spreadsheet functions, Microsoft Excel also has graphing capabilities, to make line graphs, bar charts, histograms, and scatter plots, to which you can add trend lines and automatically calculate correlation coefficients.

Students who do not have access to Microsoft products can find other solutions, such as cost-free software packages available from OpenOffice.org, or online applications such as the ones available from Google. See the hotlinks section at the end of the chapter for more information.

The sections below list the functions and capabilities of the various software programs that students should consider learning about and using in their lab reports. To find out how to use them, look through the menus of whichever program you are using. The way the lists are set out below, the first word suggests which menu or tab to start to looking in, although many programs have icons with some of the more frequently used functions. Examples have been provided for some functions.

If you cannot find a feature in a program you are using, do not hesitate to go online. Do a search for 'how do I ...?' and type in the function you are looking for, and finish the search with 'in ...' and type in the name of the software and the version. If it is important that the solution is specifically for Mac, say so in your search otherwise there is a good chance the solutions you find will be for non-Mac users. For example, 'How do I insert footnotes in Microsoft Word for Mac?'

Word processing

Students should know how to do the following things with a word processor.

- Format: changing text formatting, such as putting species names in *italics*.
- Format: turning numbers into subscripts (e.g. H_2O) and superscripts (e.g. cm^3).
- Table: setting up tables, merging cells, aligning text horizontally/vertically within cells, rotating text 90°.
- Table/ruler/tabs: aligning decimal points within columns of a table.
- Table: adding borders around the cells so that they show up clearly.
- Insert: adding bulleted lists and numbered lists.

- Insert: adding a photo and resizing it to fit, and including a legend with the photo. Students should know how to adjust the quality or resolution of images to avoid the problem of the file size of their documents being too big. This can be a particular issue when submitting a document electronically.
- Edit: pasting a graph copied from a graphing program; if the lab report is going to be submitted electronically, is best to paste the graph as an image rather than as a linked object.
- Insert: using shapes such as arrows or boxes to annotate an image.
- Insert: adding notes such as footnotes at the bottom of a page, or endnotes at the end of the document.
- Insert: adding formulas using formula editors to produce well-presented equations to show how you processed your data. Note that some versions of word processors do not have the formula editors pre-installed so they need to be added manually.
- Insert: adding symbols such as ±, Δ, λ, or ≤ where necessary.
- Insert: using page breaks to avoid having a section start at the bottom of a page or to avoid having a table split over two pages.
- Tools: selecting the text and setting the proofing language for the language you are using.
- Edit: using paste special for pasting text or numbers without the formatting.

Spreadsheets

Students should know how to do the following things with a spreadsheet program.

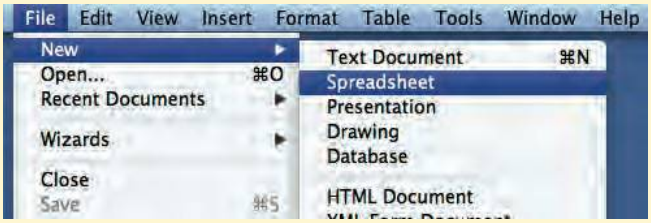
- Understand the system of identifying cells as A1, B2, C3, etc. (see screenshot 2 on the next page).
- Format: changing the format of the cell to match the type of data, such as number, date, percentage, text, time, scientific notation, etc.
- Format: changing the number of decimal places after the decimal point to correspond to the desired degree of precision.
- Insert: using math operations by inserting an equals sign '=' followed by a formula using 'A1 + A2' to add, or 'B3/B2' to divide, or '(A1 + A2 + A3)*B1' to combine more than one operation in the same formula.
- Insert: inserting predefined formulas such as sum, average, maximum or minimum, standard deviation, chi-squared, etc. Example for Excel: typing '=max(A1:A100)' in cell A101 finds the maximum value between A1 and A100. Replacing the term 'max' with 'min' in the formula finds the minimum value. Note: if your software is installed in a language other than English, the commands may be different. For example, 'sum' is 'somme' in French versions of spreadsheet software.
- For a repeating operation, copying the formula down a column or across a row rather than re-typing it separately each time.
- Converting a relative reference into an absolute reference by adding $, for example B2 does not behave the same way as $B2 or B$2 or B2 when it is copied and pasted to another place on the sheet.
- With international settings, the decimal point can sometimes be a full point (.) and sometimes be a comma (,) so if the decimals in your data do not seem to be recognized by the program, it is possible that you need to switch from one to the other. Instead of doing this manually, use the find and replace feature in the edit menu.

Worked example

Use a spreadsheet program to calculate the mean, mode, and median of the data mentioned earlier in the chapter.

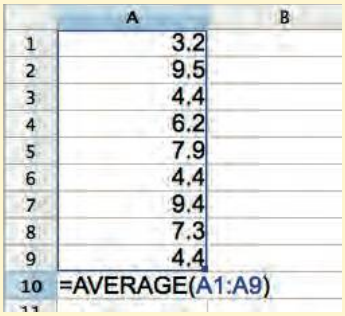
Solution

Be sure a spreadsheet program is installed on the computer or tablet you are using, such as Microsoft Excel or the spreadsheet programs available in software packages such as NeoOffice, LibreOffice, and Apache OpenOffice. The screenshots in this chapter are from OpenOffice Calc, which is available at no cost online.



Screenshot 1. Creating a new spreadsheet in OpenOffice.

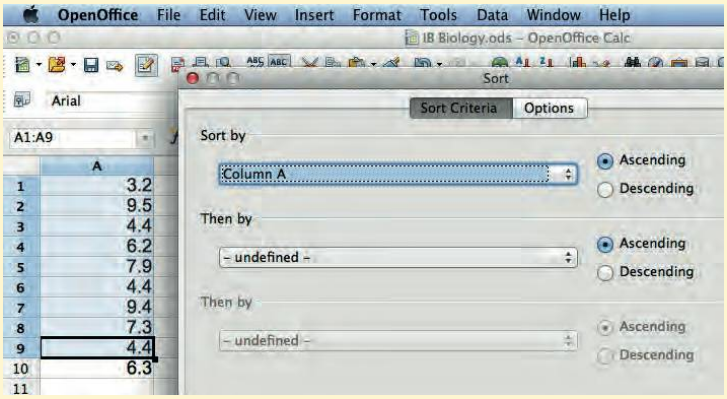
By typing in the values into the cells A1–A9 of your spreadsheet, you can then enter a formula to calculate the mean in cell A10. See screenshot 2.



Screenshot 2. Calculating the mean (average) by using the '=average' function and either selecting cells A1–A9 by manually selecting them or typing A1:A9 in the parentheses. When you hit the ENTER key, it should calculate 6.3.

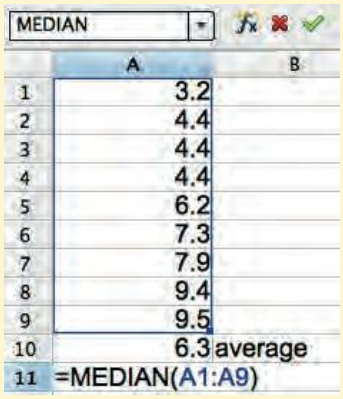
If you get an error message, be sure your program is not expecting a comma (,) instead of a full point (.) for the decimal point. In certain international versions of spreadsheet programs, the default is for a comma.

To find the median in the spreadsheet program, first select the nine values, then go to the Data menu and select Sort.



Screenshot 3. Using the sort feature to put numbers in order. Notice how cell A10 is purposely left out of the selection, as it is not part of the data (it is the calculated mean).

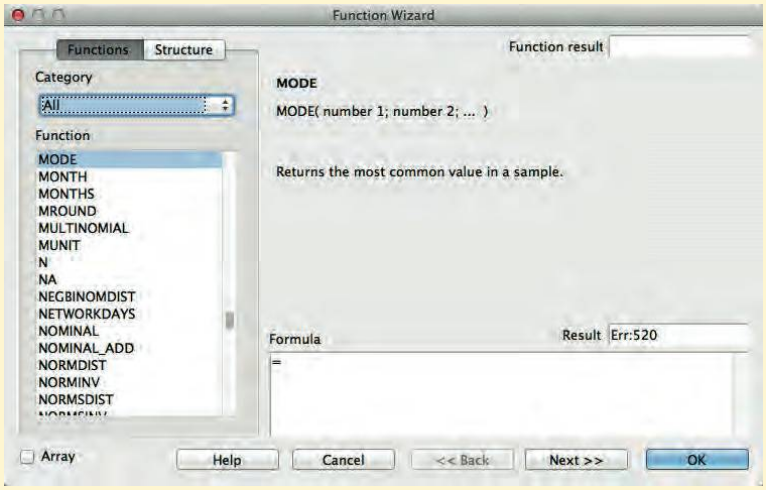
You could find the median manually by counting the data points and finding the one in the middle, or you could let the computer calculate it: type in the formula '=median(' and select the data between A1 and A9.



Screenshot 4. Using other formulas for the data.

Note that the median function works even if you do not sort the data as we did in screenshot 3.

Screenshot 5 shows finding the mode.



Screenshot 5. By selecting 'Function' from the 'Insert' menu, you can choose from a long list of possible calculations to perform. Here, 'MODE' is shown, but there are many others, such as MAX, MIN, etc. As for the other functions, use the range of data A1 to A9 in the parentheses. Microsoft Excel and other spreadsheet programs have similar lists of functions to insert.

Graphing

Students should know how to do the following things with graphing software.

- Entering the data in proper columns and rows so that the computer recognizes the data with its headings.
- Defining which data will be graphed by carefully selecting the correct rows and lines (it is important that there are no blank rows or blank columns in the selected data).
- Insert: selecting a type of graph that will lead to useful analysis.
- Insert: once the data points on a scatter plot are selected, a trend line can be inserted.
- Options: once the data points on the graph are selected and a trend line added, graphing programs often suggest options such as inserting the formula for the trend line or calculating the r^2-value.
- Options: once data points on the graph are selected, error bars can be added.

- Format: adding a title to the graph as well as labels to the axes. Many graphing programs suggest a legend, but legends are only necessary if two or more colours are used.
- Options: Once the numbers on the *x*- or *y*-axis are selected, it is often possible to alter the maximum and minimum values (useful for zooming in on a part of the graph that is interesting) or changing the scale (sometimes it is clearer to show every fifth value or every tenth value rather than every number on the scale).
- Options: for graphs showing two values for *y* measured in two different units, it is sometimes necessary to add a second *y*-axis using a different scale to avoid the problem of one variable's graph being squashed and unreadable.

As with all skills, it will probably take some time to learn the software the first time you use it. But with practice and perseverance, you should become proficient. Learning these skills will undoubtedly help you in your future studies after IB, and many will help you later in your career.

Worked example

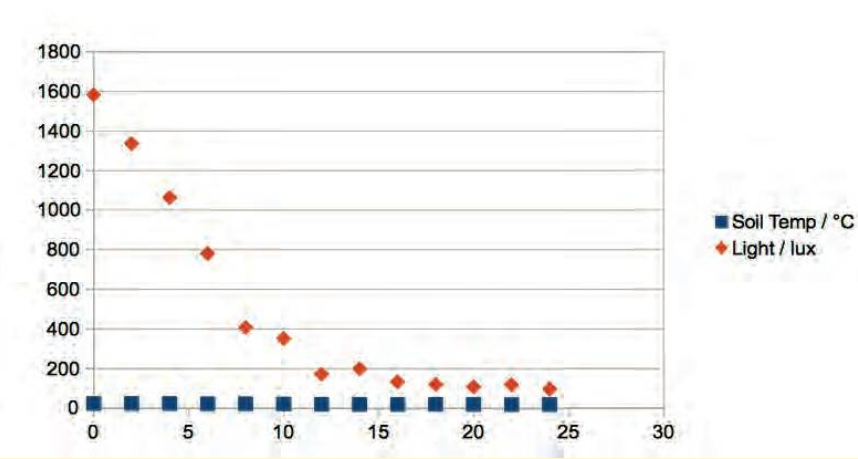
Make a scatter plot graph using the following data points of abiotic factors measured by students doing fieldwork between an open grassy area (towards the 0 m side) and a woodland (towards the 24 m side).

	A	B	C
1	**Distance / m**	**Soil Temp / °C**	**Light / lux**
2	0	22.5	1582
3	2	23.0	1336
4	4	22.4	1063
5	6	21.4	780
6	8	21.0	407
7	10	20.2	351
8	12	18.4	171
9	14	18.2	198
10	16	17.8	132
11	18	18.3	119
12	20	17.8	107
13	22	16.7	117
14	24	17.4	95

Screenshot 6. The raw data.

Solution

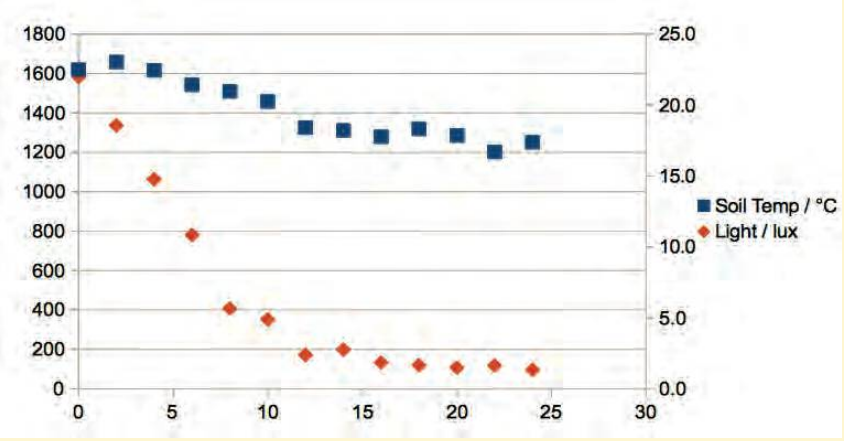
First, enter the data as shown in columns A, B, and C. Second, select the cells A1–C14: it is recommended that you include the labels of the data in row 1 when graphing data. Note: too many students look at data like this and decide to do two separate graphs, one for soil temperature and another for light levels. However, it saves space and allows a better comparison if both variables are graphed together. Third, indicate to your spreadsheet program that you want to insert a graph. Fourth, choose the graph type, in this case a scatter plot, in order to plot *y* against *x*.



Screenshot 7. A graph that is not very useful.

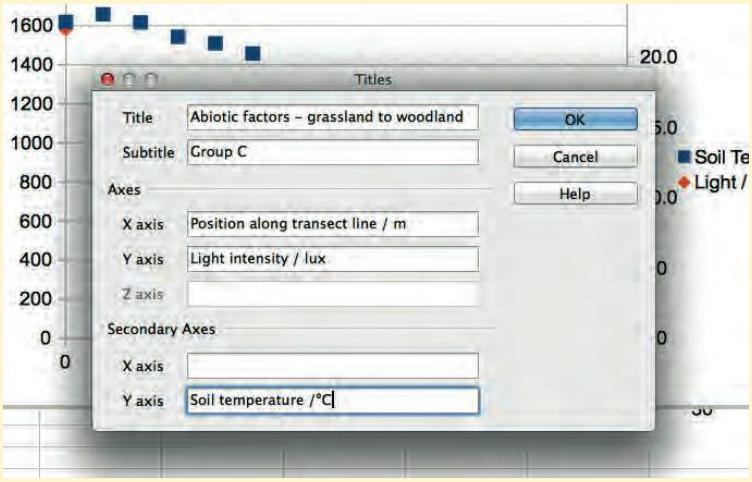
What you have at this point (screenshot 7) is far from satisfactory, and you need to work through quite a few options in order to obtain a graph that will allow you to analyse the trends.

By selecting the blue data points (the soil temperature), it is possible to right-click on them and ask OpenOffice to change the format of the data series. Select 'secondary Y axis' for the blue data points. This will create a second *y*-axis on the right.



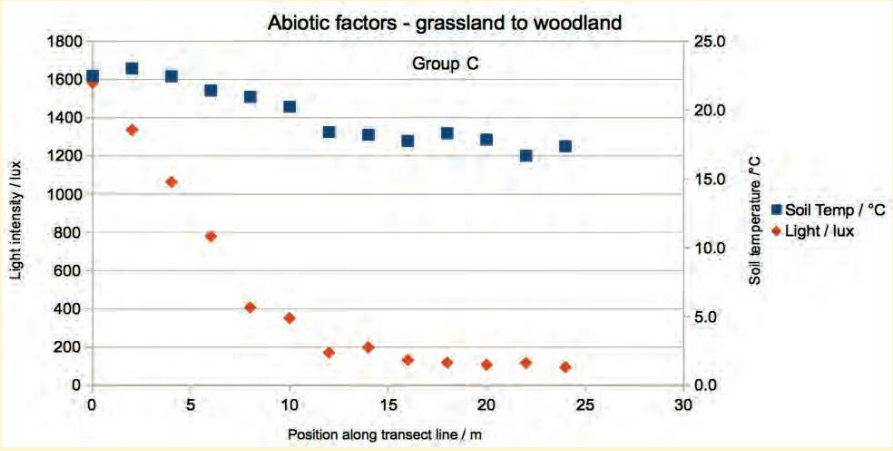
Screenshot 8. Creating a secondary *y*-axis.

This solves the problem of not being able to see any changes in the soil temperature in °C because it is on the same scale as the light readings in lux. But the graph is still not finished. To add a title to the graph and labels on the axes, make sure the graph is selected (by double-clicking on it) and choose 'Titles' from the options.



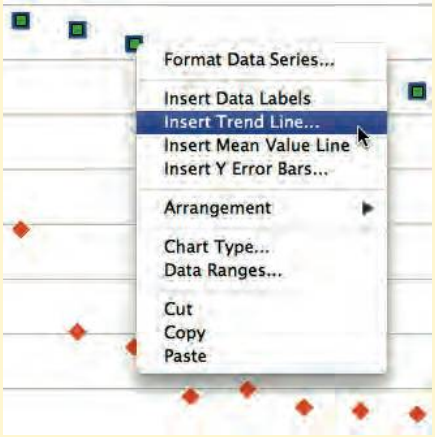
Screenshot 9. Adding a title and labels for the axes.

Now the graph should look like screenshot 10.



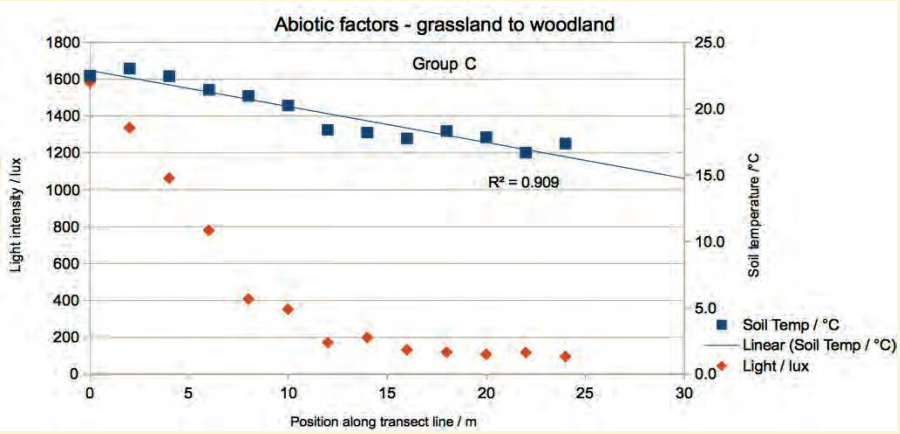
Screenshot 10. Labels added.

To finish data processing, a trend line could be added to one or both sets of data points. Here is an example of a trend line being added to the soil temperature by right-clicking on the selected data points.



Screenshot 11. Using a drop-down menu to insert a trend line.

The r^2-value can been added by right-clicking on the selected trend line and choosing the format options. Do not be surprised if the graphing software writes r^2 as R^2.



Screenshot 12. The graph is now ready to interpret.

There is a trend in the temperature that goes from warmer temperatures in the open grassland near the 0 m mark, and cooler temperatures in the woodland towards the 24 m mark. The light levels seem to be a good indication of where the tree line starts to block out the sunlight after 10 m. This graph might inspire you to see the correlation from 0 to 10 m and compare it to 11 to 20 m.

To learn more about maths and ICT skills, go to the hotlinks site, search for the title or ISBN and click on Mathematics, and information and communication technology skills.

Just because you spend time making a graph look great does not mean it is worth including in a lab report. Sometimes it will inspire you to look for other patterns. In this example, further processing could be done by plotting soil temperature against light levels to see whether they are correlated. It is advisable that you not wait until the night before an assignment on data processing is due before learning how to graph. Remember when you were a child and you first learned how to tie your shoelaces? Remember how long it took the first time? This is true for many skills and preparing graphs for data processing is no exception. The first few times you make a graph will take a long time. Once you are an expert, it will take much less time.

Here is a closing thought about your smartphone: have you ever considered using it as a measuring device for lab work? Smartphones have microphones to measure sound levels, accelerometers that can be used for detecting vibrations or measuring angles as a spirit level, a camera that can be used as a lux meter or to make slow motion videos, a GPS that can measure your position and many other things. Check out app stores online for the ones available for your phone. Ideas include a click counter, decibel meter, timer, tape measure, 'radar' speed gun, and many more. When doing microscope work in the lab, certain smartphone cameras work quite well for taking photos of what you are observing. Different teachers have different philosophies about the use of smartphones in the lab or during fieldwork but it is a shame not to take advantage of this powerful computer in your pocket. Whether or not you become a scientist later in life, the maths and ICT skills you learn in the IB will help you throughout your life.

The biology extended essay

One of the requirements of the IB diploma is to write an extended essay. This in-depth study of a limited topic within a particular subject area provides you with the chance to carry out independent research within a subject of your choosing. This essay is restricted to 4000 words and is expected to involve about 40 hours of work. Most schools introduce this requirement during the last portion of the first year of the IB programme. The essay is then completed and handed in during the second year.

The extended essay is awarded marks according to a very specific set of criteria and, in conjunction with the theory of knowledge (TOK) essay, can contribute to your overall diploma score through the award of bonus points. These points can be quite helpful in gaining your IB diploma. If excellent marks are obtained on both essays, you may be awarded a bonus of three points. However, if you do very badly in both essays, you will not be eligible for an IB diploma.

One of the subjects often selected for the extended essays is biology. It is particularly popular because many of the topics discussed in class and researched in laboratory activities provide unique ideas for the essay. Most successful extended essays in biology involve experimental work. However, some literature-based essays have scored well. Many submitted essays include both approaches.

An important requirement of the extended essay is that it should represent a new or unique approach to addressing a specific research question. Creativity and an individualistic approach are important to achieve a high mark. The table that follows offers you some guidance on writing your essay. However, there is no set formula that guarantees success in this important requirement.

Before you start, you must be aware of the criteria on which your essay will be marked. You will be assigned a supervisor (a teacher at your school) who will be available to discuss your progress with you. He or she will also help you ensure the safety and appropriateness of your work.

Advice on criteria for assessing your extended essay

Criterion	Advice
Research question	A good research question is essential to a good extended essay. It should be stated early in the introduction and should be focused for a 4000-word essay. Adequate time and thought must be spent in writing the research question.
Introduction	This should provide an explanation of the research question. The introduction should include discussion of the significance of the research question.
Investigation	The procedure is the key. Is it unique? Does it allow for adequate data collection? Are controls used? Is the procedure truly biological in nature? Is the procedure relevant to the research question? If the paper is library based, this criterion involves a detailed look at how the data to be analysed were obtained.

Criterion	Advice
Knowledge and understanding of the topic studied	Is an academic context evident? Have you shown that you clearly understand all aspects of the essay? Do your analyses represent an obvious understanding?
Reasoned argument	In your quest to confirm your hypothesis, are you logical and methodical in your approach and explanation? Is a convincing argument presented?
Application of analytical and evaluative skills	If library based, has there been careful analysis of all sources? Have all aspects of the experiment been evaluated for appropriateness? Is the presentation of data logical? Has there been adequate data analysis?
Use of language appropriate to the subject	Is the language appropriate to the topic and is it correctly used? Is it clear and precise? Does the terminology represent understanding?
Conclusion	Does the conclusion flow logically from the arguments in the essay? Is the conclusion relevant to the research question and does it relate to the original hypothesis? Does the conclusion include unresolved questions and potential future research?
Formal presentation	This includes elements such as a title page, table of contents, page numbers, appropriate illustrations, proper citations and bibliography, and appropriate appendices, if used.
Abstract	This is written last and includes three elements: • the research question • the investigative approach • a conclusion. • Is it within 300 words?
Holistic judgement	This criterion is used to reward creative and unique approaches. It also involves depth of understanding, insight, and apparent interest in the topic.

These criteria are the key to a strong extended essay. It is important to remember that a biology extended essay must be clearly related to biology. Ethical considerations are extremely important and should be discussed with your supervisor. Discuss all aspects of your procedure with your supervisor before beginning, so that safety issues are not a concern.

The emphasis on this essay is independent research. Select a topic that is interesting to you and do substantial library research before beginning the process of writing the research question and formulating a hypothesis. Obtain samples of past successful extended essays from your supervisor for basic ideas on how to approach the task ahead.

The completion of your extended essay is followed by a viva voce (concluding interview). This is a 10–15 minute interview with your supervisor. It provides an opportunity to reflect on successes and on what has been learned.

Enjoy your research.

Suggestions for course study and strategies for the IB biology exam

To be successful as an IB biology student, you should achieve the following.

- Develop a body of knowledge that characterizes the concepts of biology, and the methods and techniques necessary to carry out basic experimentation in the subject.
- Demonstrate the ability to apply this body of knowledge in explanations, analyses, and evaluations.
- Communicate this knowledge and ability in proper, acceptable ways.
- Appreciate the ethical aspects of our subject area, as well as an understanding of the possibilities and limitations of biology and science in general.
- Utilize mathematical skills accurately and efficiently. These skills include basic mathematical functions, data table preparation and understanding, graph plotting and interpretation, and basic statistical analysis.

These suggestions/requirements will involve a great deal of dedication on your part. It will be essential to listen carefully and participate fully in your class activities. Practical experiences (laboratory activities) are essential in your full development as an IB biology student.

General suggestions for course study

The following is a list of study habits that have helped many students become successful in biology.

1. Study at the same time and place every day until it becomes automatic.
2. Pick an area to study in that is well lit and uncluttered, to minimize distractions.
3. Use a method to keep track of major assignment and test dates. This will help avoid the need for last minute emergency cramming.
4. Start your study sessions with the most difficult material first, then go to the easier materials. End the study session with a quick review.
5. By including several subjects in your study sessions, you may avoid loss of concentration on one particular subject.
6. Be as active as possible in your study. For example, you could include outlining, re-wording, condensing, and reciting facts aloud. Discussions with partners and groups are often beneficial.
7. Take notes on main ideas in class discussions and from reading to help you concentrate and grasp the material better.
8. Leave space when taking notes on paper to add information when reviewing the information at a later time.
9. Re-read and revise your notes as soon as possible after taking them. This means the information is fresh in your mind, and it is easier to complete or edit phrases.
10. Mnemonic devices (assisting the memory) are extremely effective. Make up a sentence or word with the same initials as the material you need to memorize or understand.

11 Break up larger projects into smaller tasks spread over many days. This helps make the project less intimidating.
12 Always strive to see the 'big picture'. Try to see the material you learn each day as part of the whole picture of biology. Realize that each day's material is a small segment of the interconnected parts that comprise biology.
13 Complete assignments and any class work as neatly as possible. Make certain you order materials as they are covered in class. One suggestion is to consider your notebook as a publishable work of knowledge!
14 Always begin test preparation early. This gives you time to seek help if necessary. It is more effective to study in short sessions (perhaps 20–30 minutes) than in long single study sessions, such as the infamous 'all-nighters'.
15 Have a designated plan for each study session. Do not wander aimlessly through your studies.
16 Choose appropriate sources on the internet to supplement your biology knowledge.
17 Note or flash cards are a highly recommended form of study at any level. It involves active learning that is highly effective in gaining knowledge.
18 Plan physical activity, recreation, and quiet time for yourself, as well as your studies.

Specific suggestions for IB biology course study using this text

This book has been designed to help you achieve success in IB biology. It provides guidance to allow you to fulfil the suggestions/requirements presented earlier in this section. Some key features to note include the following.

1 Essential ideas are presented at the beginning of each chapter. These should focus your thoughts on the general concepts to be discussed in the chapters.
2 Statements labelled Nature of science are included at the beginning of each section. These allow you to grasp the overarching theme in the study of all the major sciences. They often present major improvements in scientific investigations that occurred during the 21st century.
3 Understandings and Applications and skills are extremely important and are clearly presented at the beginning of each section.
4 Understandings present a detailed description of the knowledge you should achieve. It is vital to study the text and e-text information carefully so that these Understandings can be mastered. Background building is essential to master these statements.
5 Application and skills statements outline the specific applications and skills to be developed from the understandings. These statements represent higher levels of thought and are often quite demanding.
6 When you come across Hints for success in the book, take special note. These present activities or suggestions that will make you a better IB biology student.
7 The extracts marked International mindedness present essential material that may be very helpful in the exams.
8 Utilization information and lab or practical activities will be of great value in being successful in the IB exams.

9 Other sections/features presented in the book that should be carefully noted for course success include Key points, Animations, Theory of knowledge, hotlinks, and Interesting information.

By focusing on these features during the course and as a revision activity near the exam, you will become a much more effective IB biology student.

Strategies for success when answering questions in the IB exams

IB exams for both standard level (SL) and higher level (HL) biology are dominated by four types of questions:

- multiple-choice questions
- data-based questions
- open-ended questions
- short-answer questions.

Strategy for success when answering multiple-choice questions

SL exams contain 30 multiple-choice questions, and HL exams contain 40 multiple-choice questions. All multiple-choice questions have four choices (A–D) and there is no penalty for guessing. Therefore you should answer every question.

The content for multiple-choice questions comes from the core material for SL students, and the core and additional higher level (AHL) material for HL students. No material from the options appears in multiple-choice questions.

Strategies for success when answering data-based questions

Data-based questions present you with data in some form and then ask you questions about that data. Some questions will ask you to read the data displayed, and some will ask you to draw conclusions from it.

Pay close attention to the number of marks for each question. The examiner is comparing your answer to acceptable answers on a mark scheme. You can write as much as you like as long as you do not contradict yourself. Grading is positive so, if you write something wrong, no marks are deducted. But if you contradict yourself, you receive no mark.

You are expected to use the data given within the question. Make it a habit to reference the data when you practise data-based questions; this will make it natural to do the same when taking the exam. Become familiar with unit expressions such as kJ m^{-2} yr^{-1} (read as kilojoules per metre squared per year). If you are not comfortable with the unit expressions you see in data-based questions in this book, ask your instructor for help.

A glossary of 'command terms' is provided in the eBook that accompanies this text. You should be very familiar with the meaning of each command term and respond appropriately when they are used in exam questions.

If the question has the command term 'calculate', you must show your work.

Questions that use the command term 'compare' require you to relate clearly the similarities *and* differences between two sets of data. In most situations, an answer that involves numeric data will not achieve a mark unless a unit is given with the number. Do a full comparison and be sure to state whether any difference is an increase or decrease.

When encountering command terms such as 'describe' or 'outline', provide a general or big-picture summary of the data presented. Include a numerical value or values when asked to 'describe' a data pattern.

When directed to 'explain the results', be certain to write the reasons or mechanisms that produced the results.

'Suggest' questions indicate that you are to use your knowledge from throughout the course to provide causes for the data presented.

Use a ruler when answering data-based questions because the graphs are often small and the degree of precision required in your answers is often quite demanding. A ruler can be used to draw lines on the graph to help you increase your chance of being within the degree of tolerance allowed by the mark scheme.

Practise the questions given at the end of each chapter in this book. They are from past exam papers and a mark scheme is provided. Write out the answers as you would during an exam. Give yourself a set time to answer all of the questions in a section. Finally, grade yourself with the answer key (the mark scheme) provided in the eBook.

Strategies for success when answering open-ended questions

As with data-based questions, it is essential you are familiar with the meaning of the command terms when answering open-ended or extended-response questions. These types of questions often involve a larger number of possible marks than other questions.

'Explain' or 'discuss' command terms typically cannot be done with a brief response. There is never a penalty for writing too much. The problem here is that you could spend too much time answering one question at the expense of another. Look at how many marks the question is worth. If one question is worth 6 marks, it is worth spending more time on it than a question worth only 2 marks. When answering a 'discuss' question, make sure you present at least two alternative views. For example, imagine a discussion about conserving the rainforest. You must give opposing views on why the rainforest should and should not be conserved.

When a question includes the command term 'list', you must give the exact number of things asked. For example, if the question is 'List three factors that affect the distribution of plant species', you should list only three factors. If you list four, the fourth answer will not be scored. However, if a specific number is not specified, you may list more than the number of marks calls for.

Remember these tips.

- The examiner does not know you. You must communicate fully what you know and not expect the examiner to 'fill in the blanks' with information that you do not relate clearly.
- State the obvious in your answers. Many of the items in a mark scheme will be information that is very basic in relation to the question.

- Do not use abbreviations that may be unfamiliar to someone else. Be clear and concise with your choice of words.
- If you have handwriting that is very small or unclear, *print* your response. If the examiner cannot read your writing, you will not get a mark.
- Make sure to use the 'supplemental booklets' at the test site for continuing answers if you run out of space in the exam booklet. Do not write outside the boxes provided on the exam booklets. This is because the exams are being e-marked, and any writing outside the lines provided will not be visible to the examiner. Number the remainder of the response in the 'supplemental booklet' so that the examiner knows where the response continues.

If a question requires you to 'draw and label', follow these directions.

- All drawings need to be done within the boxes provided. These boxes are of adequate size to represent the complexity required.
- Coloured pens, pencils, and highlighters should not be used because the papers will be marked electronically. Use a black pencil for the drawing, and use black ink for the labels.
- Horizontal labelling is recommended.
- Use a ruler to draw a line from the label to the item in the drawing, and be sure the line touches exactly the part that it is labelling (if the examiner has any doubt about the structure that you are naming, no marks will be awarded).
- There should be no gaps in the lines when drawing closed shapes such as cells or organelles.
- Structures should be positioned correctly within the drawing. Connections between included structures within drawings should be clearly and properly shown.
- Correct proportions of structures included in a drawing is required.

Strategies for success when answering short-answer questions

Short-answer questions involve fewer marks per question than data-based or open-ended/extended-response questions. However, the command term involved is just as important with this type of question as with data-based or open-ended/extended-response questions. This type of question is often divided into parts, and is usually answerable in the few lines provided. Separate distinct ideas from one another: this decreases the chances of the examiner missing the different parts of your answer.

And finally

Remember that the three papers of the written IB exam account for 80% of your overall score. The other 20% is based on your performance in the internal assessment (lab work) portion of the course. This internal assessment portion is graded by your instructor and moderated by an examiner.

The exam papers are based on the requirements and direction presented in the course subject guide. It is therefore vital that you become very familiar with the requirements and directions that relate to the material for your level of study (SL or HL).

In the exam

1. The first day of the exam presents papers 1 and 2. These papers test your knowledge of the core material (plus AHL material for HL students). No option material is tested on the first day.
2. The second day of the exam presents paper 3. This paper tests the option material plus questions based on the experimental work carried out as directed in the course subject guide. Paper 3 will include questions from the core (also from the AHL for HL students) particularly relating to skills and techniques, and data analysis and evaluation.
3. It is highly recommended that you read each question twice before beginning to write. Examiners report a disturbing number of students who write an answer that does not correspond to the question (often indicating poor understanding of command terms). In addition, when you have finished, if time allows, re-read the questions and your answers one more time just to be certain everything is in order. Make sure you cross out clearly any work that you do not want the examiner to mark.

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HIGHER LEVEL

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HIGHER LEVEL

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