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Biology

for Cambridge International AS & A Level

COURSEBOOK

Mary Jones, Richard Fosbery, Dennis Taylor & Jennifer Gregory

Fifth edition

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Contents

Introduction

This is the fifth edition of the Cambridge International AS & A Level Biology Coursebook, and it provides everything that you need to support your course for Cambridge AS & A Level Biology (9700). It provides full coverage of the syllabus for examinations from 2022 onwards.

The chapters are arranged in the same sequence as the topics in the syllabus. Chapters 1 to P1 cover the AS material, and Chapters 12 to P2 cover the material needed for A Level. The various features that you will find in these chapters are explained on the next two pages.

Many questions will test a deeper understanding of the facts and concepts that you will learn during your course. It is therefore not enough just to learn words and diagrams that you can repeat in your examinations; you need to ensure that you really understand each concept fully. Trying to answer the questions that you will find within each chapter, and at the end of each chapter, should help you to do this.

Although you will study your biology as a series of different topics, it is very important to appreciate that all of these topics link up with each other. You need to make links between different areas of the syllabus to answer some questions. For example, you might be asked a question that involves bringing together knowledge about protein synthesis, infectious disease and transport in mammals. In particular, you will find that certain key concepts come up again and again. These include:

- Cells as units of life
- Biochemical processes
- DNA, the molecule of heredity
- Natural selection
- Organisms in their environment
- Observation and experiment.

As you work through your course, make sure that you keep reflecting on the work that you did earlier and how it relates to the current topic that you are studying. Some of the reflection questions at the ends of the chapters suggest particular links that you could think about. They also ask you to think about *how* you learn, which may help you to make the very best use of your time and abilities as your course progresses. You can also use the self-evaluation checklists at the end of each chapter to decide how well you have understood each topic in the syllabus, and whether or not you need to do more work on each one.

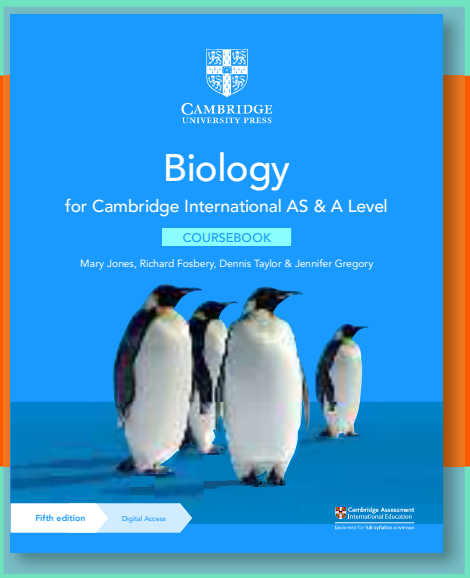
Practical skills are an important part of your biology course. You will develop these skills as you do experiments and other practical work related to the topics you are studying. Chapters P1 (for AS Level) and P2 (for A Level) explain what these skills are and what you need to be able to do.

You may like to look at two other books in this series – the Workbook and the Practical Workbook. The Workbook provides clear guidance on many of the skills that you need to develop as you work through the course – such as constructing and analysing graphs, and planning experiments – with exercises for you to try. The Practical Workbook is full of detailed explanations of how to carry out all the practicals required in the syllabus, and many others too, that will help you to become more confident in practical work.

This is an exciting time to be studying biology, with new discoveries and technologies constantly finding their way into the news. We very much hope that you will enjoy your biology course, and that this book will help you not only to prepare for your examinations but also to develop a life-long interest in this subject.

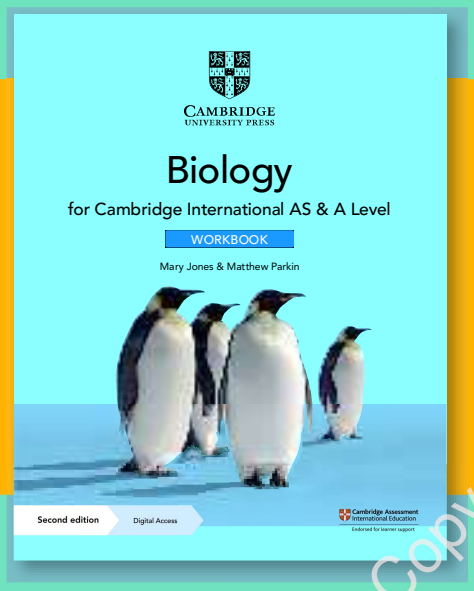
How to use this series

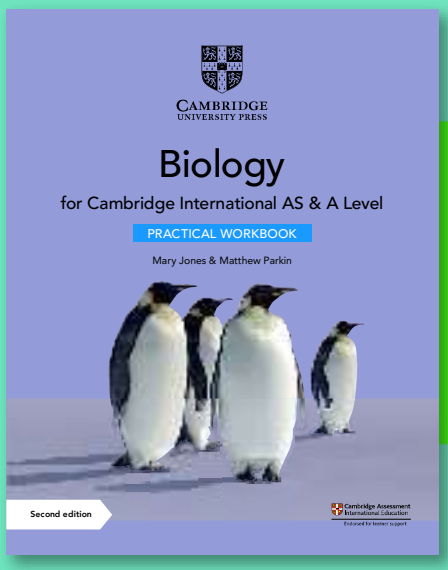
This suite of resources supports students and teachers following the Cambridge International AS & A Level Biology syllabus (9700). All of the books in the series work together to help students develop the necessary knowledge and scientific skills required for this subject. With clear language and style, they are designed for international learners.



The coursebook provides comprehensive support for the full Cambridge International AS & A Level Biology syllabus (9700). It clearly explains facts, concepts and practical techniques, and uses real-world examples of scientific principles. Two chapters provide full guidance to help students develop investigative skills. Questions within each chapter help them to develop their understanding, while exam-style questions provide essential practice.

The workbook contains over 100 exercises and exam-style questions, carefully constructed to help learners develop the skills that they need as they progress through their Biology course. The exercises also help students develop understanding of the meaning of various command words used in questions, and provide practice in responding appropriately to these.

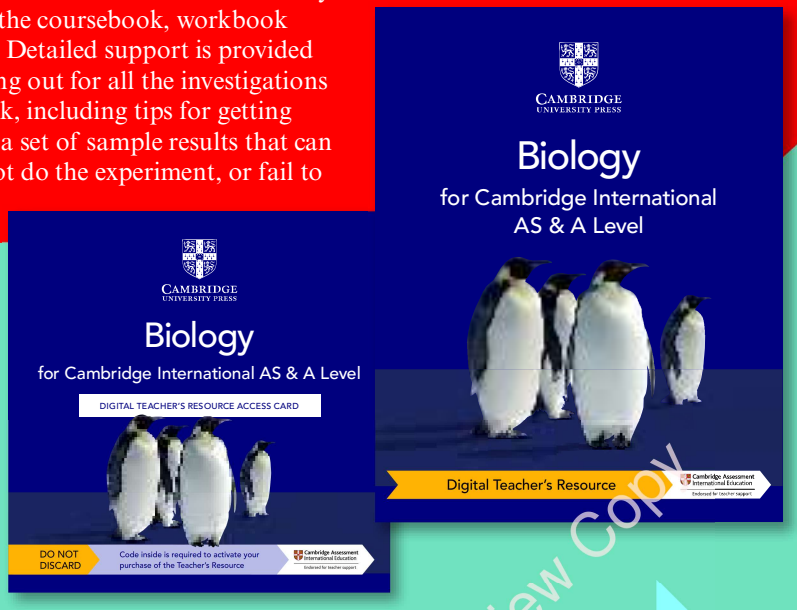




This write-in book provides students with a wealth of hands-on practical work, giving them full guidance and support that will help them to develop all of the essential investigative skills. These skills include planning investigations, selecting and handling apparatus, creating hypotheses, recording and displaying results, and analysing and evaluating data.

The teacher's resource supports and enhances the questions and practical activities in the coursebook. This resource includes detailed lesson ideas, as well as answers and exemplar data for all questions and activities in the coursebook and workbook. The practical teacher's guide, included with this resource, provides support for the practical activities and experiments in the practical workbook.

Teaching notes for each topic area include a suggested teaching plan, ideas for active learning and formative assessment, links to resources, ideas for lesson starters and plenaries, differentiation, lists of common misconceptions and suggestions for homework activities. Answers are included for every question and exercise in the coursebook, workbook and practical workbook. Detailed support is provided for preparing and carrying out for all the investigations in the practical workbook, including tips for getting things to work well, and a set of sample results that can be used if students cannot do the experiment, or fail to collect results.



How to use this book

Throughout this book, you will notice lots of different features that will help your learning. These are explained below.

LEARNING INTENTIONS

These set the scene for each chapter, help with navigation through the coursebook and indicate the important concepts in each topic.

BEFORE YOU START

This contains questions and activities on subject knowledge you will need before starting this chapter.

SCIENCE IN CONTEXT

This feature presents real-world examples and applications of the content in a chapter, encouraging you to look further into topics. There are discussion questions at the end which look at some of the benefits and problems of these applications.

PRACTICAL ACTIVITY

This book does not contain detailed instructions for doing particular experiments, but you will find background information about the practical work you need to do in these boxes. There are also two chapters, P1 and P2, which provide detailed information about the practical skills you need to develop during the course.

Questions

Appearing throughout the text, questions give you a chance to check that you have understood the topic you have just read about. You can find the answers to these questions in the digital version of the Coursebook.

KEY WORDS

Key vocabulary is highlighted in the text when it is first introduced. Definitions are then given in the margin, which explain the meanings of these words and phrases.

You will also find definitions of these words in the Glossary at the back of this book.

COMMAND WORDS

Command words that appear in the syllabus and might be used in exams are highlighted in the exam-style questions when they are first introduced. In the margin, you will find the Cambridge International definition. You will also find these definitions in the Glossary at the back of the book with some further explanation on the meaning of these words.*

*The information in this section is taken from the Cambridge International syllabus (9700) for examination from 2022. You should always refer to the appropriate syllabus document for the year of your examination to confirm the details and for more information. The syllabus document is available on the Cambridge International website at www.cambridgeinternational.org.

WORKED EXAMPLE

Wherever you need to know how to use a formula to carry out a calculation, there are worked examples boxes to show you how to do this.

REFLECTION

These activities ask you to look back on the topics covered in the chapter and test how well you understand these topics and encourage you to reflect on your learning.

IMPORTANT

Important equations, facts and tips are given in these boxes.

EXAM-STYLE QUESTIONS

Questions at the end of each chapter provide more demanding exam-style questions, some of which may require use of knowledge from previous chapters. Some questions are taken from past papers. Where this is the case, they include references to the relevant past paper. All other questions are written by the authors. Answers to these questions can be found in the digital version of the Coursebook.

SUMMARY

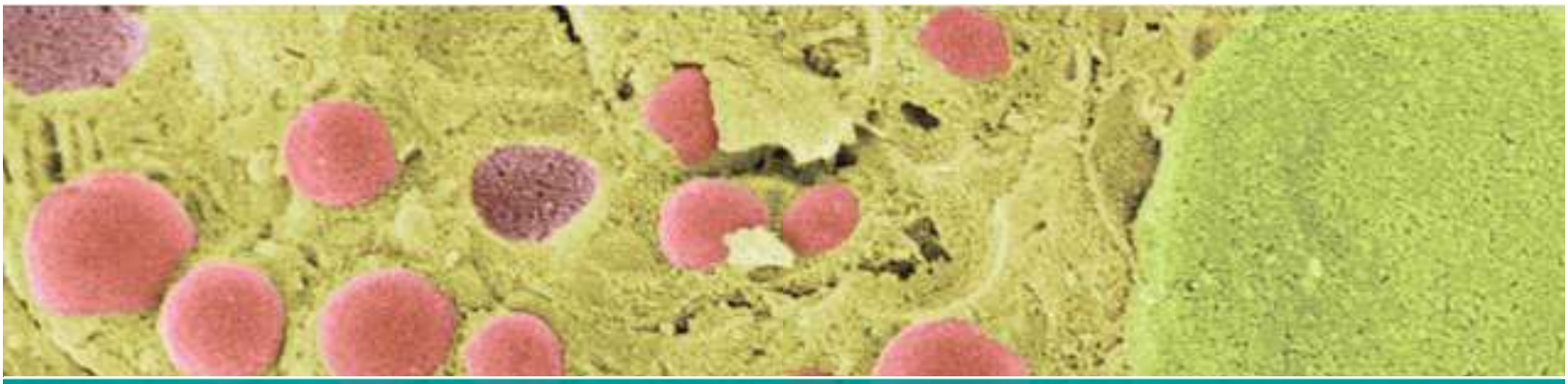
There is a summary of key points at the end of each chapter.

SELF-EVALUATION

The summary checklists are followed by 'I can' statements which match the Learning intentions at the beginning of the chapter. You might find it helpful to rate how confident you are for each of these statements when you are revising. You should revisit any topics that you rated 'Needs more work' or 'Almost there'.

I can	See section	Needs more work	Almost there	Ready to move on

These boxes tell you where information in the book is extension content, and is not part of the syllabus.



Chapter 1
Cell structure

LEARNING INTENTIONS

In this chapter you will learn how to:

- explain that cells are the basic units of life
- use the units of measurement relevant to microscopy
- recognise the common structures found in cells as seen with a light microscope and outline their structures and functions
- compare the key structural features of animal and plant cells
- use a light microscope and make temporary preparations to observe cells
- recognise, draw and measure cell structures from temporary preparations and micrographs
- calculate magnifications of images and actual sizes of specimens using drawings or micrographs
- explain the use of the electron microscope to study cells with reference to the increased resolution of electron microscopes
- recognise the common structures found in cells as seen with an electron microscope and outline their structures and functions
- outline briefly the role of ATP in cells
- describe the structure of bacteria and compare the structure of prokaryotic cells with eukaryotic cells
- describe the structure of viruses.

BEFORE YOU START

- Make a list of structures that could be found in a cell.
- Try to write down the functions of the structures you have listed.
- Which structures are found in plant cells and which are found in animal cells?
- Are there any cells that are not animal or plant cells?

THINKING OUTSIDE THE BOX

Progress in science often depends on people thinking 'outside the box' – original thinkers who are often ignored or even ridiculed when they first put forward their radical new ideas. One such individual, who battled constantly throughout her career to get her ideas accepted, was the American biologist Lynn Margulis (1938–2011; Figure 1.1). Her greatest achievement was to use evidence from microbiology to help firmly establish an idea that had been around since the mid-19th century – that new organisms can be created from combinations of existing organisms. Importantly, the existing organisms are not necessarily closely related. The organisms form a symbiotic partnership (they live together in a partnership in which both partners benefit). Margulis imagined that one organism engulfed ('ate') another. Normally the engulfed organism would be digested and killed, but sometimes the organism engulfed may survive and even be of benefit to the organism in which it finds itself. This type of symbiosis is known as endosymbiosis ('endo' means inside). A completely new type of organism is created, representing a dramatic evolutionary change.

The best-known example of Margulis' ideas is her suggestion that mitochondria and chloroplasts were originally free-living bacteria (prokaryotes). She suggested that these bacteria invaded the ancestors of modern eukaryotic cells, which are much larger and more complex cells than bacteria, and entered into a symbiotic relationship with the cells. This idea has been confirmed as true by later work. Margulis saw such symbiotic unions as a major driving cause of evolutionary change. Throughout her life, she continued to challenge the traditional view, first put forward by Charles Darwin, that evolution occurs mainly as a result of competition between species.

Figure 1.1: Lynn Margulis: 'My work more than didn't fit in. It crossed the boundaries that people had spent their lives building up. It hits some 30 sub-fields of biology, even geology.'

Questions for discussion

- Can you think of any ideas people have had which were controversial at the time but are now accepted? Try to think of scientific examples. You may also like to consider why the ideas were controversial.
- Can you think of any scientific ideas people have now which are controversial and not accepted by everybody?

1.1 Cells are the basic units of life

Towards the middle of the 19th century, scientists made a fundamental breakthrough in our understanding of how life 'works'. They realised that the basic unit of life is the **cell**.

The origins of this idea go back to the early days of microscopy when an English scientist, Robert Hooke, decided to examine thin slices of plant material. He chose cork as one of his examples. Looking down the microscope, he made a drawing to show the regular appearance of the structure, as you can see in Figure 1.2. In 1665 he published a book containing this drawing.

Figure 1.2: Drawing of cork cells published by Robert Hooke in 1665.

If you examine the drawing you will see the regular structures that Hooke called 'cells'. Each cell appeared to be an empty box surrounded by a wall. Hooke had discovered and described, without realising it, the fundamental unit of all living things.

Although we now know that the cells of cork are dead, Hooke and other scientists made further observations of cells in *living* materials. However, it was not until almost 200 years later that a general cell theory emerged from the work of two German scientists. In 1838 Schleiden, a botanist, suggested that all plants are made of cells. A year later Schwann, a zoologist, suggested the same for animals. It was soon also realised that all cells come from pre-existing cells by the process of cell division. This raises the obvious question of where the original cell came from. There are many hypotheses, but we still have no definite answers to this question.

Why cells?

A cell can be thought of as a bag in which the chemistry of life occurs. The activity going on inside the cell is therefore separated from the environment outside the cell. The bag, or cell, is surrounded by a thin membrane. The membrane is an essential feature of all cells because it controls exchange between the cell and its environment. It can act as a barrier, but it can also control movement of materials across the membrane in both directions. The membrane is therefore described as partially permeable. If it were freely permeable, life could not exist, because the chemicals of the cell would simply mix with the surrounding chemicals by diffusion and the inside of the cell would be the same as the outside.

Two types of cell

During the 20th century, scientists studying the cells of bacteria and of more complex organisms such as plants and animals began to realise that there were two fundamentally different kinds of cells. Some cells were very simple, but some were much larger and more complex. The complex cells contained a **nucleus** (plural: **nuclei**) surrounded by two membranes. The genetic material, DNA, was in the nucleus. In the simple cells the DNA was not surrounded by membranes, but apparently free in the cytoplasm.

KEY WORDS

cell: the basic unit of all living organisms; it is surrounded by a cell surface membrane and contains genetic material (DNA) and cytoplasm containing **organelles**

organelle: a functionally and structurally distinct part of a cell, e.g. a ribosome or mitochondrion

nucleus (plural: **nuclei**): a relatively large organelle found in eukaryotic cells, but absent from prokaryotic cells; the nucleus contains the cell's DNA and therefore controls the activities of the cell; it is surrounded by two membranes which together form the nuclear envelope

Organisms made of cells with membrane-bound nuclei are now known as **eukaryotes**, while the simpler cells lacking membrane-bound nuclei are known as **prokaryotes** ('eu' means true, 'karyon' means nucleus, 'pro' means before). Eukaryotes are thought to have evolved from prokaryotes more than two billion years ago. Prokaryotes include bacteria. Eukaryotes include animals, plants, fungi and some other organisms.

KEY WORDS

eukaryote: an organism whose cells contain a nucleus and other membrane-bound organelles

prokaryote: an organism whose cells do not contain a nucleus or any other membrane-bound organelles

1.2 Cell biology and microscopy

The study of cells has given rise to an important branch of biology known as cell biology. Cell biologists study cells using many different methods, including the use of various types of microscope.

There are two fundamentally different types of microscope: the light microscope and the electron microscope. Both use a form of radiation in order to see the specimen being examined. The light microscope uses light as a source of radiation, while the electron microscope uses electrons, for reasons which are discussed later.

Units of measurement

In order to measure objects in the microscopic world, we need to use very small units of measurement, which are unfamiliar to most people. Before studying light and electron microscopy further, you need to become familiar with these units.

According to international agreement, the International System of Units (SI units) should be used. In this system, the basic unit of length is the metre (symbol, m). More units are created by going a thousand times larger or smaller. Standard prefixes are used for the units. For example, the prefix 'kilo' means 1000 times. Thus, 1 kilometre = 1000 metres. The units of length relevant to cell studies are shown in Table 1.1.

The smallest structure visible with the human eye is about 50–100 µm in diameter (roughly the diameter of the sharp end of a pin). The cells in your body vary in size from about 5 µm to 40 µm. It is difficult to imagine how small these cells are, especially when they are clearly visible using a microscope. An average bacterial cell is about 1 µm across. One of the smallest structures you will study in this book is the ribosome, which is only about 25 nm in diameter! You could line up about 20 000 ribosomes across the full stop at the end of this sentence.

1.3 Plant and animal cells as seen with a light microscope

Microscopes that use light as a source of radiation are called light microscopes. Figure 1.3 shows how the light microscope works.

Note: the structure of a light microscope is extension content, and is not part of the syllabus.

Fraction of a metre	Unit	Symbol
one thousandth = 0.001 = 1/1000 = 10^{-3}	millimetre	mm
one millionth = 0.000 001 = 1/1000 000 = 10^{-6}	micrometre	µm
one thousand millionth = 0.000 000 001 = 1/1000 000 000 = 10^{-9}	nanometre	nm

Table 1.1: Units of measurement relevant to cell studies: 1 micrometre is a thousandth of a millimetre; 1 nanometre is a thousandth of a micrometre.

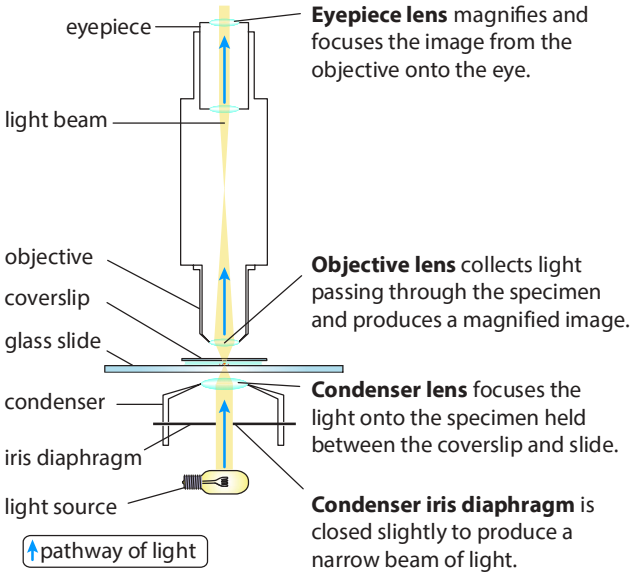


Figure 1.3: How the light microscope works. The coverslip is a thin sheet of glass used to cover the specimen. It protects specimens from drying out and also prevents the objective lens from touching the specimen.

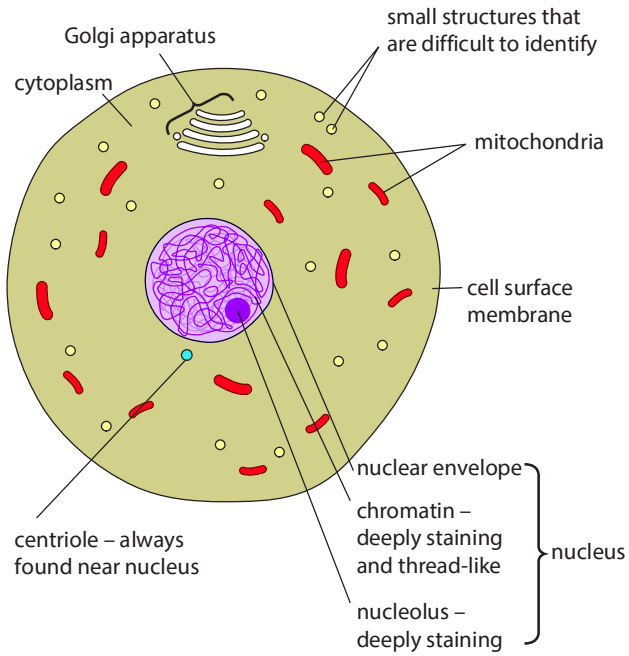


Figure 1.4: Structure of a generalised animal cell (diameter about 20 µm) as seen with a very high quality light microscope.

Figure 1.4 is a drawing showing the structure of a generalised animal cell and Figure 1.5 is a drawing showing the structure of a generalised plant cell, both as seen with a light microscope. (A generalised cell shows all the structures that may commonly be found in a cell.) Figures 1.6 and 1.7 are photomicrographs. A photomicrograph is a photograph of a specimen as seen with a light microscope. Figure 1.6 shows some human cells. Figure 1.7 shows a plant cell taken from a leaf. Both figures show cells magnified 400 times, which is equivalent to using the high-power objective lens on a light microscope. See also Figures 1.8a and 1.8b for labelled drawings of these figures.

Many of the cell contents are colourless and transparent so they need to be stained with coloured dyes to be seen. The human cells in Figure 1.6 have been stained. The chromatin in the nuclei is particularly heavily stained. The plant cells in Figure 1.5 have not been stained because the chloroplasts contain the green pigment chlorophyll and are easily visible without staining.

Question

1 Using Figures 1.4 and 1.5, name the structures that:
   - **a** animal and plant cells have in common
   - **b** are found only in plant cells
   - **c** are found only in animal cells.

Features that animal and plant cells have in common

Cell surface membrane

All cells, including those of both eukaryotes and prokaryotes, are surrounded by a very thin **cell surface membrane**. This is also sometimes referred to as the plasma membrane. As mentioned before, it is partially permeable and controls the exchange of materials between the cell and its environment.

Nucleus

All eukaryotic cells contain a nucleus. The nucleus is a relatively large structure. It stains intensely and

KEY WORD

cell surface membrane: a very thin membrane (about 7 nm diameter) surrounding all cells; it is partially permeable and controls the exchange of materials between the cell and its environment

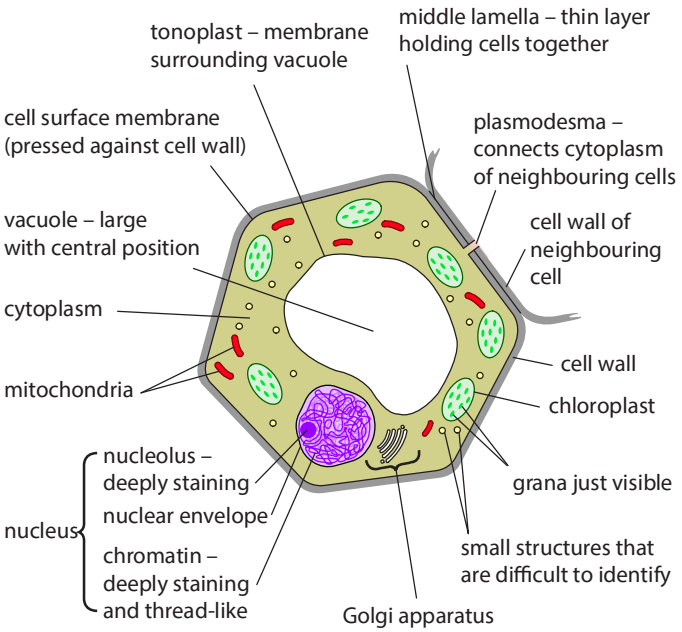


Figure 1.5: Structure of a generalised plant cell (diameter about 40 μm) as seen with a very high quality light microscope.

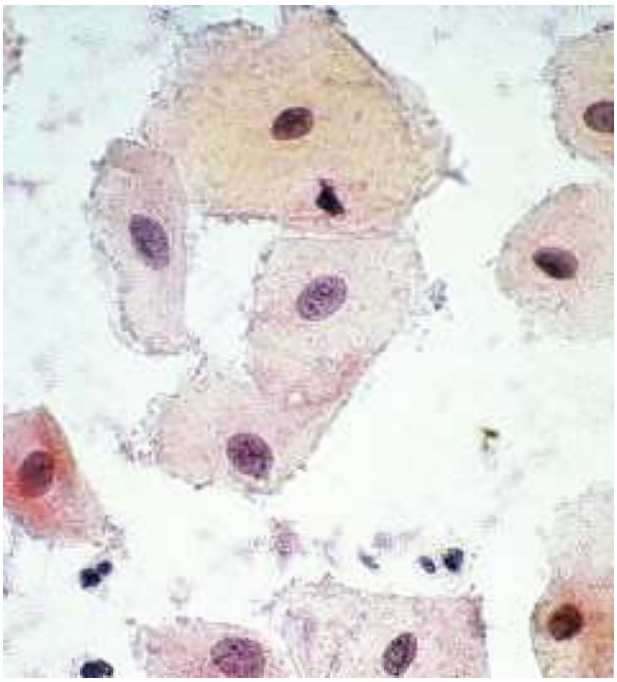


Figure 1.6: Cells from the lining of the human cheek (×400). Each cell shows a centrally placed nucleus, which is typical of animal cells. The cells are part of a tissue known as squamous (flattened) epithelium.

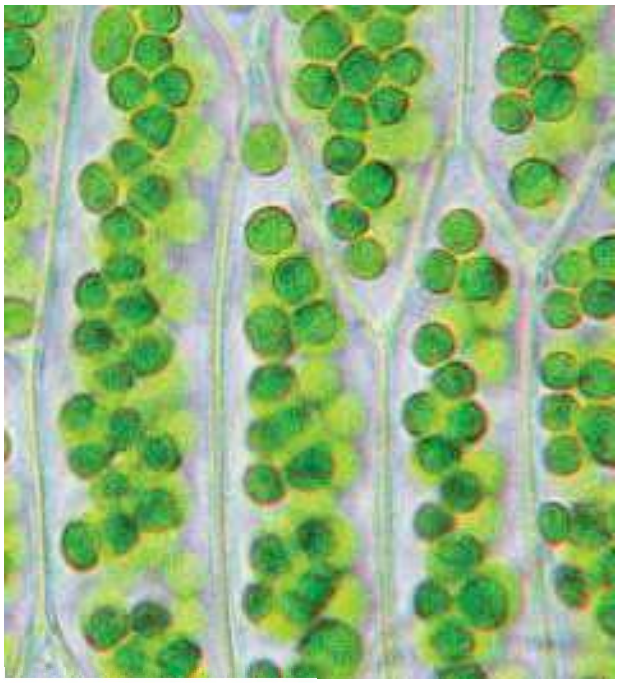


Figure 1.7: Cells in a moss leaf (×400). Many green chloroplasts are visible inside each cell. The grana are just visible as black grains inside the chloroplasts ('grana' means grains). Cell walls are also clearly visible (animal cells lack cell walls).

is therefore very easy to see when looking down the microscope. The deeply staining material in the nucleus is called **chromatin** ('chroma' means colour). Chromatin is a mass of coiled threads. The threads are seen to collect together to form **chromosomes** during nuclear division (Chapter 5, Section 5.2, Chromosomes). Chromatin contains DNA (deoxyribonucleic acid), the molecule which contains the instructions (genes) that control the activities of the cell (Chapter 6).

Inside the nucleus an even more deeply staining area is visible, the **nucleolus**. This is made of loops of DNA from several chromosomes. The number of nucleoli is variable, one to five being common in mammals. One of the main functions of nucleoli is to make ribosomes.

Cytoplasm

All the living material inside the cell is called **protoplasm**. It is also useful to have a term for all the living material outside the nucleus; it is called **cytoplasm**. Therefore, cytoplasm + nucleus = protoplasm.

Cytoplasm is an aqueous (watery) material, varying from a fluid to a jelly-like consistency. Using a light microscope, many small structures can be seen within it. These have been likened to small organs and are therefore known as organelles (meaning 'little organs'). An organelle can be defined as a functionally and structurally distinct part of a cell. Organelles are often, but not always, surrounded by one or two membranes so that their activities can be separated from the surrounding cytoplasm. Organising cell activities in separate compartments is essential for a structure as complex as an animal or plant cell to work efficiently.

Mitochondria (singular: mitochondrion)

The most numerous organelles seen with the light microscope are usually **mitochondria** (singular: **mitochondrion**). Mitochondria are only just visible using a light microscope. Videos of living cells, taken with the aid of a light microscope, have shown that mitochondria can move about, change shape and divide. They are specialised to carry out aerobic respiration.

Golgi apparatus

The use of special stains containing silver resulted in the Golgi apparatus being discovered in 1898 by Camillo Golgi. The Golgi apparatus collects and processes molecules within the cell, particularly proteins.

Note: you do not need to learn this structure. It is sometimes called the Golgi body or Golgi complex.

KEY WORDS

chromatin: the material of which chromosomes are made, consisting of DNA, proteins and small amounts of RNA; visible as patches or fibres within the nucleus when stained

chromosome: in the nucleus of the cells of eukaryotes, a structure made of tightly coiled chromatin (DNA, proteins and RNA) visible during cell division; the term 'circular DNA' is now also commonly used for the circular strand of DNA present in a prokaryotic cell

nucleolus: a small structure, one or more of which is found inside the nucleus; the nucleolus is usually visible as a densely stained body; its function is to manufacture ribosomes using the information in its own DNA

protoplasm: all the living material inside a cell (cytoplasm plus nucleus)

cytoplasm: the contents of a cell, excluding the nucleus

mitochondrion (plural: **mitochondria**): the organelle in eukaryotes in which aerobic respiration takes place

cell wall: a wall surrounding prokaryote, plant and fungal cells; the wall contains a strengthening material which protects the cell from mechanical damage, supports it and prevents it from bursting by osmosis if the cell is surrounded by a solution with a higher water potential

Differences between animal and plant cells

One of the structures commonly found in animal cells which is absent from plant cells is the centriole. Plant cells also differ from animal cells in possessing cell walls, large permanent vacuoles and chloroplasts.

Centrioles

Under the light microscope the centriole appears as a small structure close to the nucleus (Figure 1.4). Centrioles are discussed later in this chapter.

Cell walls and plasmodesmata

With a light microscope, individual plant cells are more easily seen than animal cells. This is because they are usually larger and, unlike animal cells, are surrounded by a **cell wall**. Note that the cell wall is an extra

structure which is outside the cell surface membrane. The wall is relatively rigid because it contains fibres of cellulose, a polysaccharide which strengthens the wall. The cell wall gives the cell a definite shape. It prevents the cell from bursting when water enters by osmosis, allowing large pressures to develop inside the cell (Chapter 4, Section 4.5, Movement of substances across membranes). Cell walls may be reinforced with extra cellulose or with a hard material called lignin for extra strength (Chapter 7). Cell walls are freely permeable, allowing free movement of molecules and ions through to the cell surface membrane.

Plant cells are linked to neighbouring cells by means of pores containing fine strands of cytoplasm. These structures are called **plasmodesmata** (singular: **plasmodesma**). They are lined with the cell surface membrane. Movement through the pores is thought to be controlled by the structure of the pores.

Vacuoles

Vacuoles are sac-like structures which are surrounded by a single membrane. Although animal cells may possess small vacuoles such as phagocytic vacuoles (Chapter 4, Section 4.5, Movement of substances across membranes), which are temporary structures, mature plant cells often possess a large, permanent, central vacuole. The plant vacuole is surrounded by a membrane, the **tonoplast**, which controls exchange between the vacuole and the cytoplasm. The fluid in the vacuole is a solution of pigments, enzymes, sugars and other organic compounds (including some waste products), mineral salts, oxygen and carbon dioxide.

In plants, vacuoles help to regulate the osmotic properties of cells (the flow of water inwards and outwards) as well as having a wide range of other functions. For example, the pigments which colour the petals of certain flowers and the parts of some vegetables, such as the red pigment of beetroots, may be found in vacuoles.

Chloroplasts

Chloroplasts are organelles specialised for the process of **photosynthesis**. They are found in the green parts of the plant, mainly in the leaves. They are relatively large organelles and so are easily seen with a light microscope. It is even possible to see tiny 'grains' or **grana** (singular: **granum**) inside the chloroplasts using a light microscope (Figure 1.7). These are the parts of the chloroplast that contain chlorophyll, the green pigment which absorbs light during the process of photosynthesis. Chloroplasts are discussed further in Chapter 13 (Section 13.2, Structure and function of chloroplasts).

KEY WORDS

plasmodesma (plural: **plasmodesmata**): a pore-like structure found in plant cell walls; plasmodesmata of neighbouring plant cells line up to form tube-like pores through the cell walls, allowing the controlled passage of materials from one cell to the other; the pores contain ER and are lined with the cell surface membrane

vacuole: an organelle found in eukaryotic cells; a large, permanent central vacuole is a typical feature of plant cells, where it has a variety of functions, including storage of biochemicals such as salts, sugars and waste products; temporary vacuoles, such as phagocytic vacuoles (also known as phagocytic vesicles), may form in animal cells

tonoplast: the partially permeable membrane that surrounds plant vacuoles

chloroplast: an organelle, bounded by an envelope (i.e. two membranes), in which photosynthesis takes place in eukaryotes

photosynthesis: the production of organic substances from inorganic ones, using energy from light

grana (singular: **granum**): stacks of membranes inside a chloroplast

IMPORTANT

- You can think of a plant cell as being very similar to an animal cell but with extra structures.
- Plant cells are often larger than animal cells, although cell size varies enormously.
- Do not confuse the cell wall with the cell surface membrane. Cell walls are relatively thick and physically strong, whereas cell surface membranes are very thin. Cell walls are freely permeable, whereas cell surface membranes are partially permeable. All cells have a cell surface membrane, but animal cells do not have a cell wall.
- Vacuoles are not confined to plant cells; animal cells may have small vacuoles, such as phagocytic vacuoles, although these are not usually permanent structures.

PRACTICAL ACTIVITY 1.1

Making temporary slides

A common method of examining material with a light microscope is to cut thin slices of the material called 'sections'. The advantage of cutting sections is that they are thin enough to allow light to pass through the section. The section is laid ('mounted') on a glass slide and covered with a coverslip to protect it. Light passing through the section produces an image which can then be magnified using the objective and eyepiece lenses of the microscope.

Biological material may be examined live or in a preserved state. Prepared slides contain material that has been killed and preserved in a life-like condition.

Temporary slides are quicker and easier to prepare and are often used to examine fresh material containing living cells. In both cases the sections are typically stained before being mounted on the glass slide.

Temporary preparations of fresh material are useful for quick preliminary investigations. Sometimes macerated (chopped up) material can be used, as when examining the structure of wood (xylem). A number of temporary stains are commonly used. For example, iodine in potassium iodide solution is useful for plant specimens. It stains starch blue-black and will also colour nuclei and cell walls a pale yellow. A dilute solution of methylene blue can be used to stain animal cells such as cheek cells.

Viewing specimens yourself with a microscope will help you to understand and remember structures. Your understanding can be reinforced by making a pencil drawing on good quality plain paper. Remember always to draw what you see, and not what you think you should see.

Procedure

Place the biological specimen on a clean glass slide and add one or two drops of stain. Carefully lower a cover over the specimen to protect the microscope lens and to help prevent the specimen from drying out. Adding a drop of glycerine and mixing it with the stain can also help prevent drying out.

- Suitable animal material: human cheek cells obtained by gently scraping the lining of the cheek with a finger nail
- Suitable plant material: onion epidermal cells, lettuce epidermal cells, *Chlorella* cells, moss slip leaves

(See Practical Investigation 1.1 in the Practical Workbook for additional information.)

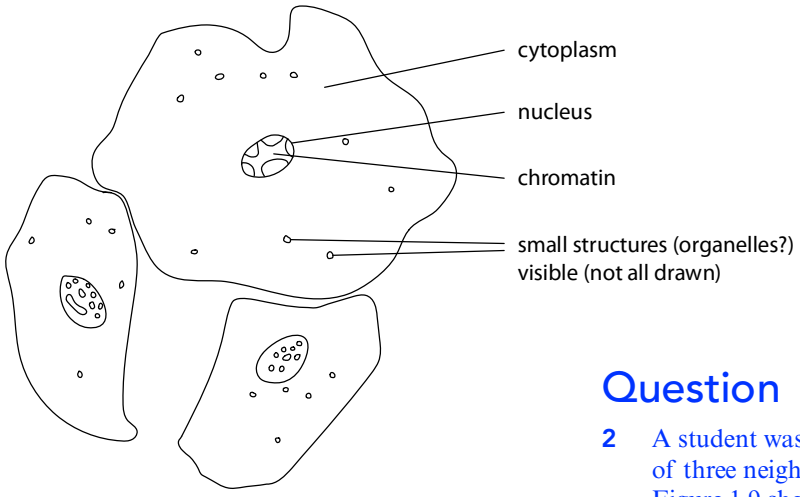
PRACTICAL ACTIVITY 1.2

Biological drawing

To reinforce your learning, you will find it useful to make labelled drawings of some of your temporary and permanent slides, as well as labelled drawings of photomicrographs.

Practical Activity 7.1 in Chapter 7 provides general guidance on biological drawing. Read the relevant sections of Practical Activity 7.1 before answering the question below, which is relevant to this chapter. Figures 1.8a and b show examples of good drawing and labelling technique based on Figures 1.6 and 1.7. Note that it is acceptable to draw only a representative portion of the cell contents of Figure 1.7, but add a label explaining this.

a



b

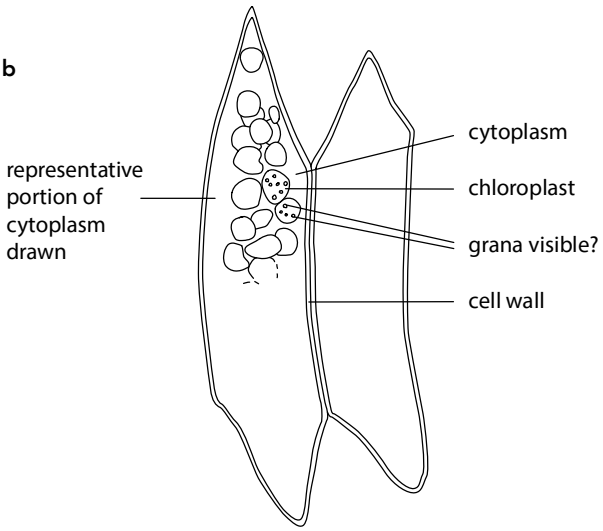


Figure 1.8: Examples of good drawing technique: **a** high-power drawing of three neighbouring animal cells from Figure 1.6; **b** high-power drawing of two neighbouring plant cells from Figure 1.7.

Question

2 A student was asked to make a high-power drawing of three neighbouring cells from Figure 1.6. Figure 1.9 shows the drawing made by the student.

Using Practical Activity 7.1 to help you, suggest how the drawing in Figure 1.9 could be improved.

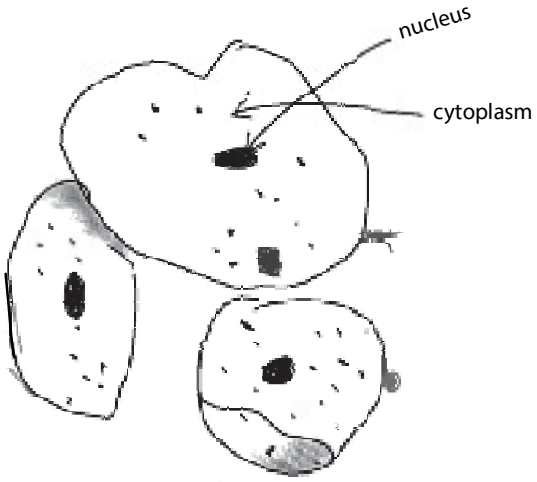


Figure 1.9: A student's high-power drawing of three neighbouring cells from Figure 1.6.

(See Practical Investigation 1.1 in the Practical Workbook for additional information.)

1.4 Measuring size and calculating magnification

Magnification is the number of times larger an image of an object is than the real size of the object.

$$\text{magnification} = \frac{\text{observed size of the image}}{\text{actual size}}$$

or

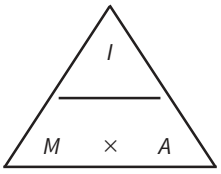
$$M = \frac{I}{A}$$

M = magnification

I = observed size of the image (what you can measure with a ruler)

A = actual size (the real size – for example, the size of a cell before it is magnified).

If you know two of the values M, I and A, you can work out the third one. For example, if the observed size of the image and the magnification are known, you can work out the actual size $A = \frac{I}{M}$. If you write the formula in a triangle as shown below and cover up the value you want to find, it should be obvious how to do the right calculation.



Measuring cell size

Cells and organelles can be measured with a microscope by means of an **eyepiece graticule**. This is a transparent scale. It usually has 100 divisions (see Figure 1.10a). The eyepiece graticule is placed in the microscope eyepiece so that it can be seen at the same time as the object to be measured, as shown in Figure 1.10b. Figure 1.10b shows the scale over one of a group of six human cheek epithelial cells (like those shown in Figure 1.6). The cell selected lies between 40 and 60 on the scale. We therefore say it measures 20 eyepiece units in diameter (the difference between 60 and 40). We will not know the actual size of the eyepiece units until the eyepiece graticule is calibrated.

KEY WORDS

magnification: the number of times larger an image of an object is than the real size of the object; magnification = image size ÷ actual (real) size of the object

eyepiece graticule: small scale that is placed in a microscope eyepiece

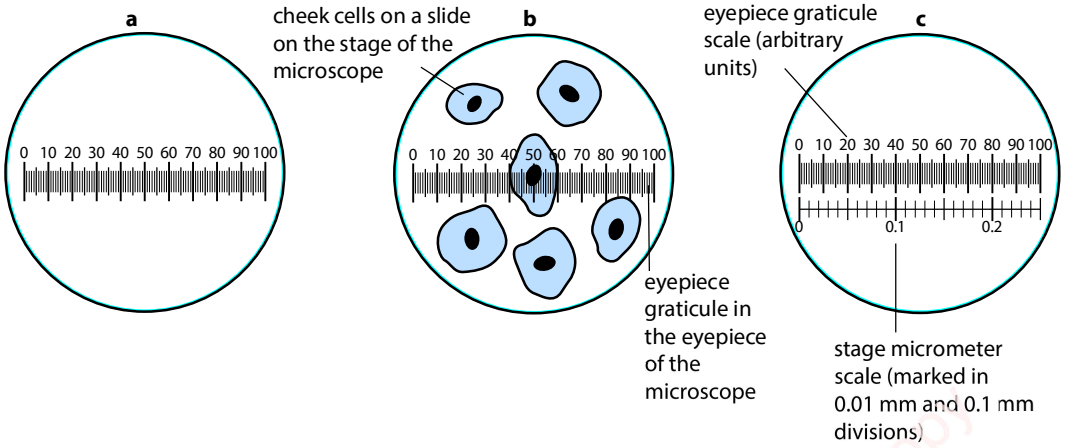


Figure 1.10: Microscopical measurement. Three fields of view seen using a high-power (×40) objective lens: **a** an eyepiece graticule scale; **b** superimposed images of human cheek epithelial cells and the eyepiece graticule scale; **c** superimposed images of the eyepiece graticule scale and the stage micrometer scale.

To calibrate the eyepiece graticule, a miniature transparent ruler called a **stage micrometer** is placed on the microscope stage and is brought into focus. This scale may be etched onto a glass slide or printed on a transparent film. It commonly has subdivisions of 0.1 and 0.01 mm. The images of the stage micrometer and the eyepiece graticule can then be superimposed (placed on top of one another) as shown in Figure 1.10c.

Calculating magnification

Figure 1.11 shows **micrographs** of two sections through the same plant cell. The difference in appearance of the two micrographs is explained in the next section.

If we know the actual (real) length of a cell in such a micrograph, we can calculate its magnification, M, using the formula:

$$M = \frac{I}{A}$$

KEY WORDS

stage micrometer: very small, accurately drawn scale of known dimensions, engraved on a microscope slide

micrograph: a picture taken with the aid of a microscope; a photomicrograph (or light micrograph) is taken using a light microscope; an electron micrograph is taken using an electron microscope

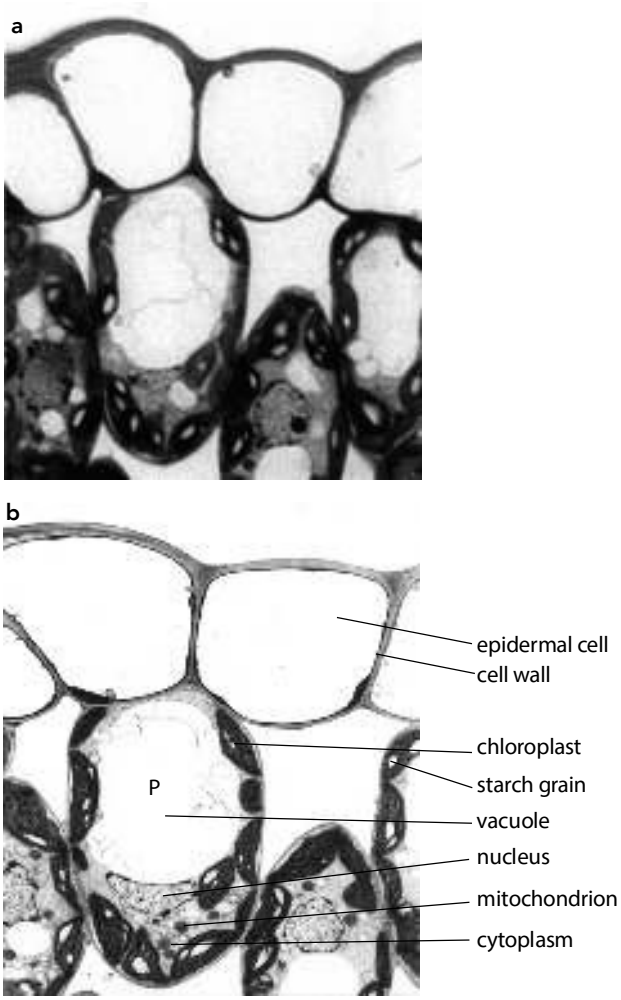


Figure 1.11: Micrographs of two sections of the same plant cells, as seen **a** with a light microscope, and **b** with an electron microscope. Both are shown at the same magnification (about ×750).

WORKED EXAMPLE

1 In the eyepiece graticule shown in Figure 1.10, 100 units measure 0.25 mm. Hence, the value of each eyepiece unit is:

$$\frac{0.25}{100} = 0.0025 \text{ mm}$$

Or, converting mm to µm:

$$\frac{0.25 \times 1000}{100} = 2.5 \text{ µm}$$

The diameter of the cell shown superimposed on the scale in Figure 1.8b measures 20 eyepiece units and so its actual diameter is:

$20 \times 2.5 \text{ µm} = 50 \text{ µm}$

This diameter is greater than that of many human cells because the cell is a flattened epithelial cell.

WORKED EXAMPLE

2 Suppose we want to know the magnification of the plant cell labelled P in Figure 1.11b. The real length of the cell is 80 µm.

Step 1 Measure the length in mm of the cell in the micrograph using a ruler. You should find that it is about 50 mm.

Step 2 Convert mm to µm. (It is easier if we first convert all measurements to the same units – in this case micrometres, µm.)

So: 1 mm = 1000 µm

50 mm = 50 × 1000 µm

= 50 000 µm

Step 3 Use the equation to calculate the magnification.

$$\text{magnification, } M = \frac{\text{image size, } l}{\text{actual size, } A}$$

$$= \frac{50\,000\ \mu\text{m}}{80\ \mu\text{m}}$$

$$= \times 625$$

The multiplication sign (×) in front of the number 625 means 'times'. We say that the magnification is 'times 625'.

Question

3 **a** Calculate the magnification of the drawing of the animal cell in Figure 1.4.

b Calculate the actual (real) length of the chloroplast labelled **X** in Figure 1.34.

WORKED EXAMPLE

3 Figure 1.12 shows a lymphocyte with a scale bar. We can use this scale bar to calculate the magnification.

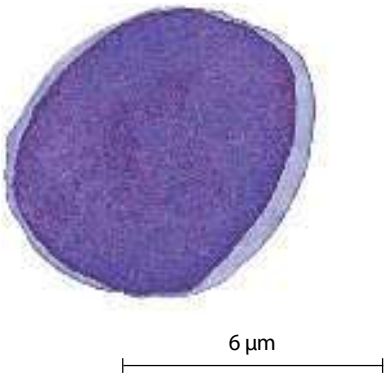


Figure 1.12: A lymphocyte.

Step 1 Measure the scale bar. Here, it is 36 mm.

Step 2 Convert mm to µm:

36 mm = 36 × 1000 µm = 36 000 µm

Step 3 The scale bar represents 6 µm. This is the actual size, A. Use the equation to calculate the magnification:

$$\text{magnification, } M = \frac{\text{image size, } l}{\text{actual size, } A}$$

$$= \frac{36\,000\ \mu\text{m}}{6\ \mu\text{m}}$$

$$= \times 6000$$

Calculating the real size of an object from its magnification

To calculate the real or actual size of an object, we can use the same magnification equation.

WORKED EXAMPLE

4 Figure 1.20 shows parts of three plant cells magnified ×5600. Suppose we want to know the actual length of the labelled chloroplast in this electron micrograph.

Step 1 Measure the observed length of the image of the chloroplast (I), in mm, using a ruler. The maximum length is 25 mm.

Step 2 Convert mm to µm:

25 mm = 25 × 1000 µm = 25 000 µm

Step 3 Use the equation to calculate the actual length:

$$\text{actual size, } A = \frac{\text{image size, } I}{\text{magnification, } M}$$

$$= \frac{25\,000\ \mu\text{m}}{5600}$$

= 4.5 µm (to one decimal place)

1.5 Electron microscopy

Before studying what cells look like with an electron microscope, you need to understand the difference between magnification and resolution.

Magnification and resolution

Look again at Figure 1.11. Figure 1.11a is a light micrograph. Figure 1.11b is an electron micrograph. Both micrographs are of the same cells and both have the same magnification. However, you can see that Figure 1.11b, the electron micrograph, is much clearer. This is because it has greater resolution. **Resolution** can be defined as the ability to distinguish between two separate points. If the two points cannot be resolved, they will be seen as one point. In practice, resolution is the amount of detail that can be seen – the greater the resolution, the greater the detail.

The maximum resolution of a light microscope is 200 nm. The reason for this is explained in the next section, 'The electromagnetic spectrum'. A resolution of 200 nm means that, if two points or objects are closer together than 200 nm, they cannot be distinguished as separate.

You might imagine that you could see more detail in Figure 1.11a by magnifying it (simply making it larger). In practice you would be able to see what is already there more easily, but you would not see any more detail. The image would just get more and more blurred as magnification increased. The resolution would not be greater.

The electromagnetic spectrum

How is resolution linked with the nature of light? One of the properties of light is that it travels in waves. The lengths of the waves of visible light vary, ranging from about 400 nm to about 700 nm. The human eye can distinguish between these different wavelengths, and in the brain the differences are converted to colour differences. Waves that are 400 nm in length are seen as violet. Waves that are 700 nm in length are seen as red.

Visible light is a form of electromagnetic radiation. The range of different wavelengths of electromagnetic radiation is called the electromagnetic spectrum. Visible light is only one part of this spectrum. Figure 1.13 shows some of the parts of the electromagnetic spectrum. The longer the waves, the lower their frequency. (All the waves travel at the same speed, so imagine them passing a post: shorter waves pass at higher frequency.) In theory, there is no limit to how short or how long the waves can be. Wavelength changes with energy: the greater the energy, the shorter the wavelength.

KEY WORD

resolution: the ability to distinguish between two objects very close together; the higher the resolution of an image, the greater the detail that can be seen

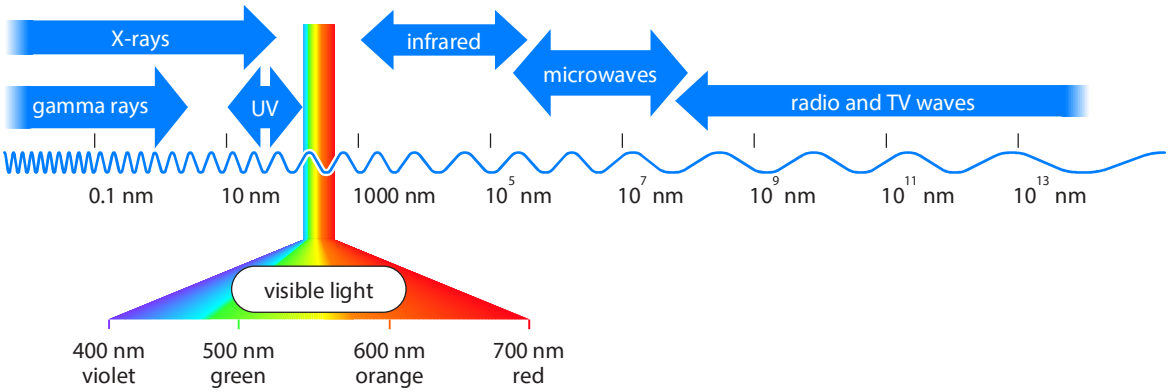


Figure 1.13: Diagram of the electromagnetic spectrum. The numbers indicate the wavelengths of the different types of electromagnetic radiation. Note the waves vary from very short to very long. Visible light is part of the spectrum. The double-headed arrow labelled UV is ultraviolet light.

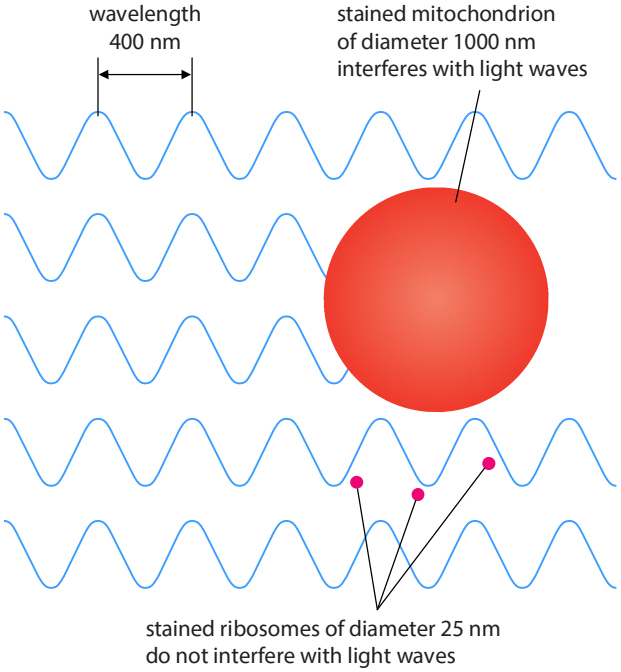


Figure 1.14: A mitochondrion and some ribosomes in the path of light waves of 400 nm length.

Now look at Figure 1.14. It shows a mitochondrion and some very small cell organelles called ribosomes. It also shows some wavy blue lines that represent light of 400 nm wavelength. This is the shortest visible wavelength. The mitochondrion is large enough to interfere with the light waves. However, the ribosomes are far too small to have any effect on the light waves.

The general rule when viewing specimens is that the limit of resolution is about one half the wavelength of the radiation used to view the specimen. In other words, if an object is any smaller than half the wavelength of the radiation used to view it, it cannot be seen separately from nearby objects. This means that the best resolution that can be obtained using a microscope that uses visible light (a light microscope) is 200 nm, since the shortest wavelength of visible light is 400 nm (violet light). Ribosomes are approximately 25 nm in diameter and can therefore never be seen using a light microscope.

If an object is transparent, it will allow light waves to pass through it and therefore will still not be visible. This is why many biological structures have to be stained before they can be seen.

Question

4 Explain why ribosomes are not visible using a light microscope.

The electron microscope

So how can we look at things smaller than 200 nm? The only solution to this problem is to use radiation of a shorter wavelength than visible light. If you study Figure 1.13, you will see that ultraviolet light or X-rays look like possible candidates. A much better solution, though, is to use electrons. Electrons are negatively charged particles which orbit the nucleus of an atom. When a metal becomes very hot, some of its electrons

gain so much energy that they escape from their orbits, similar to a rocket escaping from Earth's gravity. Free electrons behave like electromagnetic radiation. They have a very short wavelength: the greater the energy, the shorter the wavelength. Electrons are a very suitable form of radiation for microscopy for two major reasons. First, their wavelength is extremely short (at least as short as that of X-rays). Second, unlike X-rays, they are negatively charged, so they can be focused easily using electromagnets (a magnet can be made to alter the path of the beam, the equivalent of a glass lens bending light).

Using an electron microscope, a resolution of 0.5 nm can be obtained, 400 times better than a light microscope.

Transmission and scanning electron microscopes

Two types of electron microscope are now in common use. The transmission electron microscope (TEM) was the type originally developed. The beam of electrons is passed through the specimen before being viewed. Only those electrons that are transmitted (pass through the specimen) are seen. This allows us to see thin sections of specimens, and thus to see inside cells. In the scanning electron microscope (SEM), the electron beam is used to scan the surfaces of structures and only the reflected beam is observed.

An example of a scanning electron micrograph is shown in Figure 1.15. The advantage of this microscope is that surface structures can be seen. Because much of the specimen is in focus at the same time, a three-dimensional appearance is achieved. A disadvantage of the SEM is that it cannot achieve the same resolution as a TEM. Using an SEM, resolution is between 3 nm and 20 nm.

Figure 1.15: Scanning electron micrograph (SEM) of a tardigrade. Tardigrades or water bears, are about 0.5 mm long, with four pairs of legs. They are common in soil and can survive extreme environmental conditions (×86).

Viewing specimens with the electron microscope

Figure 1.16 shows how a TEM works and Figure 1.17 shows one in use.

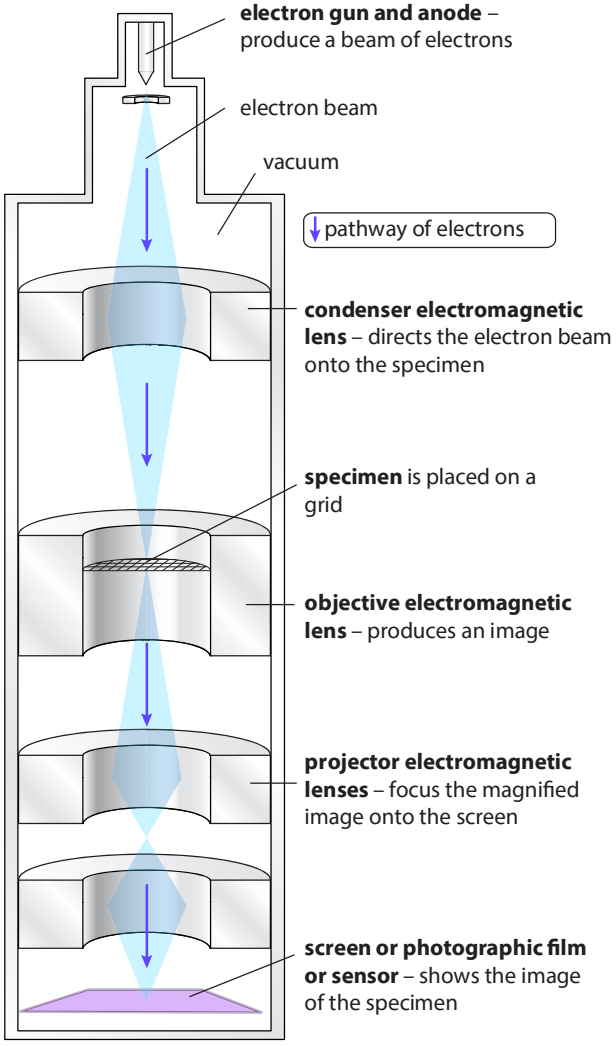


Figure 1.16: How a TEM works.

Note: the structure of an electron microscope is extension content, and is not part of the syllabus.

It is not possible to see an electron beam, so to make the image visible the electron beam has to be projected onto a fluorescent screen. The areas hit by electrons

Figure 1.17: A TEM in use.

shine brightly, giving overall a black and white picture. The stains used to improve the contrast of biological specimens for electron microscopy contain heavy metal atoms, which stop the passage of electrons. The resulting picture is like an X-ray photograph, with the more densely stained parts of the specimen appearing blacker. 'False-colour' images can be created by colouring the standard black and white image using a computer.

The electron beam, and therefore the specimen and the fluorescent screen, must be in a vacuum. If the electrons collided with air molecules, they would scatter, making it impossible to achieve a sharp picture. Also, water boils at room temperature in a vacuum, so all specimens must be dehydrated before being placed in the microscope. This means that only dead material or non-living can be examined. Great efforts are therefore made to try to preserve material in a life-like state when preparing it for electron microscopy.

1.6 Plant and animal cells as seen with an electron microscope

The fine (detailed) structure of a cell as revealed by the electron microscope is called ultrastructure and is shown in Figures 1.18–1.21.

Question

5 Copy and complete Table 1.2, which compares light microscopes with electron microscopes. Some boxes have been filled in for you.

Feature	Light microscope	Electron microscope
source of radiation		
wavelength of radiation used		*about 0.005 nm*
maximum resolution		*0.5 nm in practice*
lenses	*glass*	
specimen		*non-living or dead*
stains	*coloured dyes*	
image	*coloured*	

Table 1.2: Comparison of light microscopes and electron microscopes.

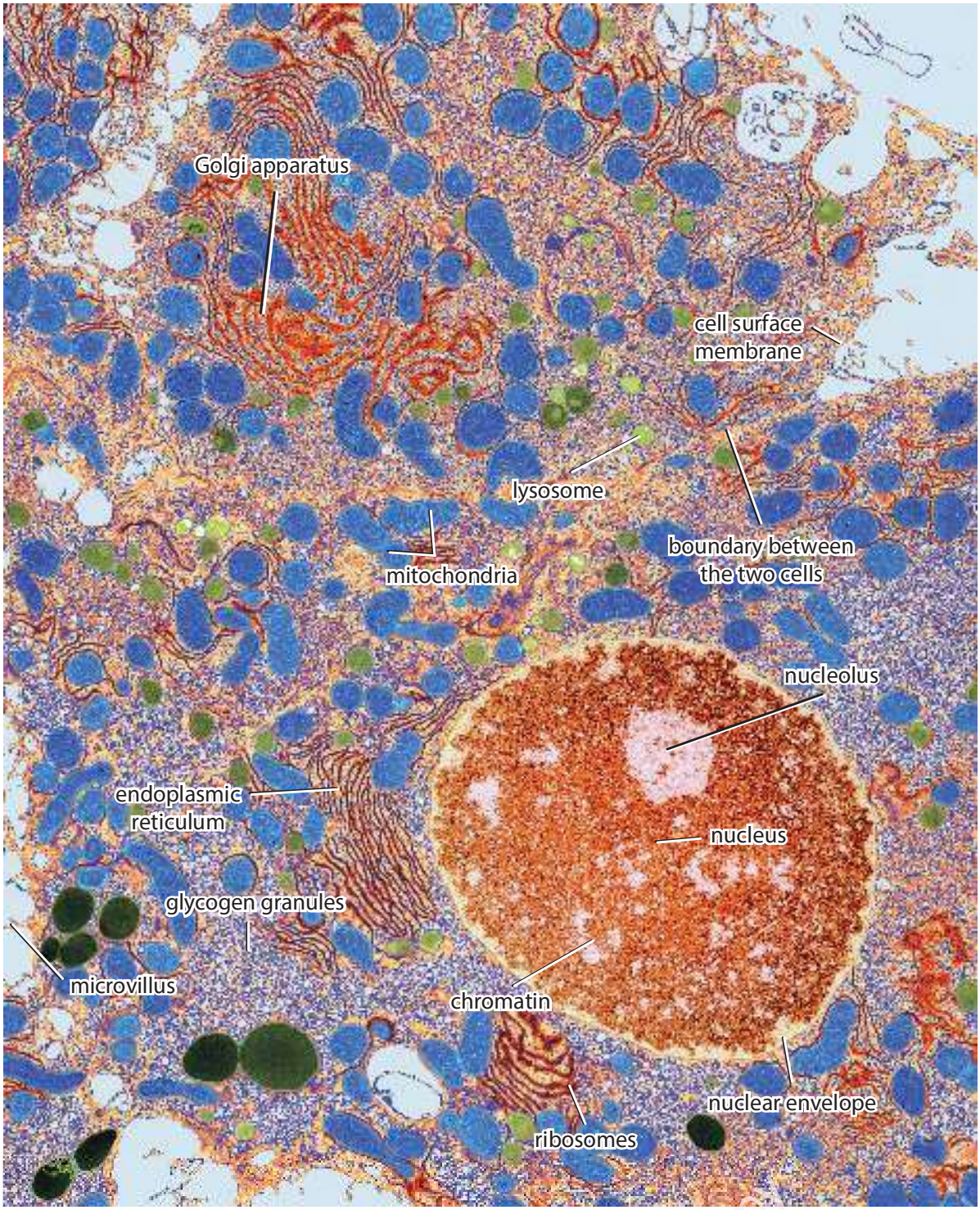


Figure 1.18: Parts of two representative animal cells as seen with a TEM. The cells are liver cells from a rat (×9600). The nucleus is clearly visible in one of the cells. The boundary between the two cells is difficult to see because the cell surface membranes are so thin.

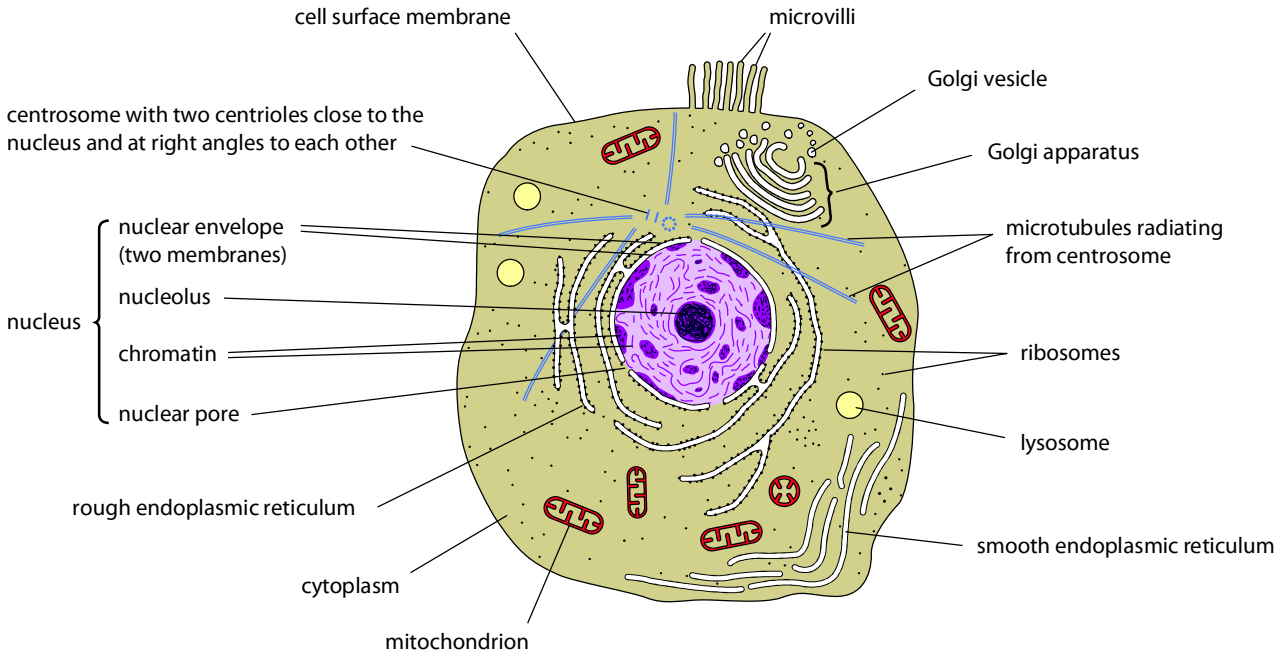


Figure 1.19: Ultrastructure of a typical animal cell as seen with an electron microscope. This drawing is based on many micrographs of animal cells. In reality, the endoplasmic reticulum is more extensive than shown here, and free ribosomes may be more extensive. Glycogen granules are sometimes present in the cytoplasm.

Question

6 Compare Figure 1.19 with Figure 1.4. Name the structures in an animal cell that can be seen with the electron microscope but not with the light microscope.

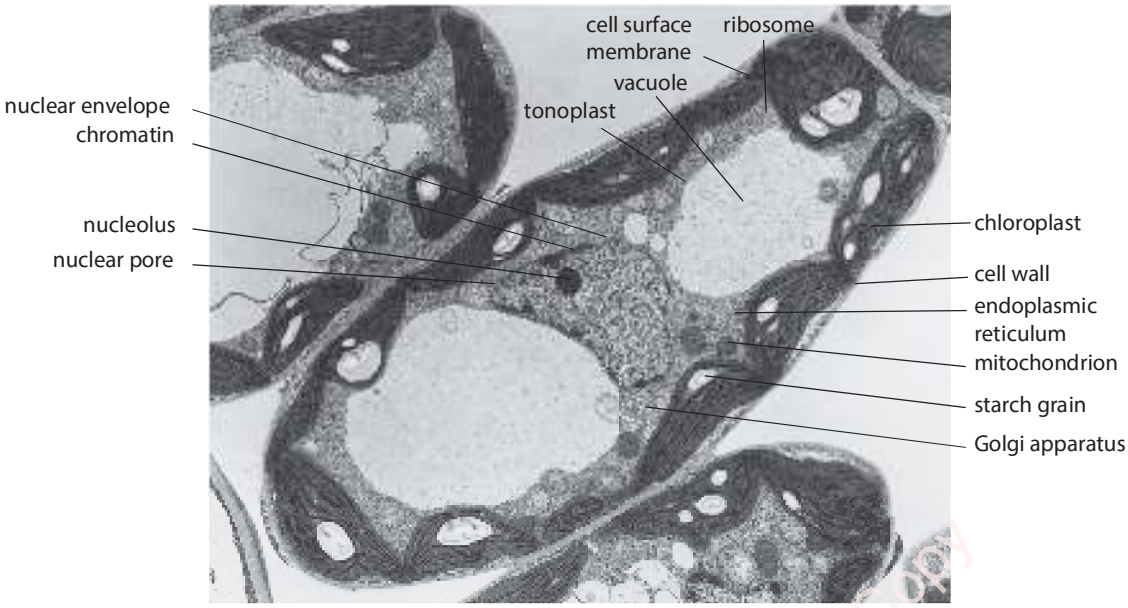


Figure 1.20: Representative plant cells as seen with a TEM. The cells are palisade cells from a soya bean leaf. The boundaries between the cells can clearly be seen due to the presence of cell walls (×5600).

Question

7 Compare Figure 1.21 with Figure 1.5. Name the structures in a plant cell that can be seen with the electron microscope but not with the light microscope.

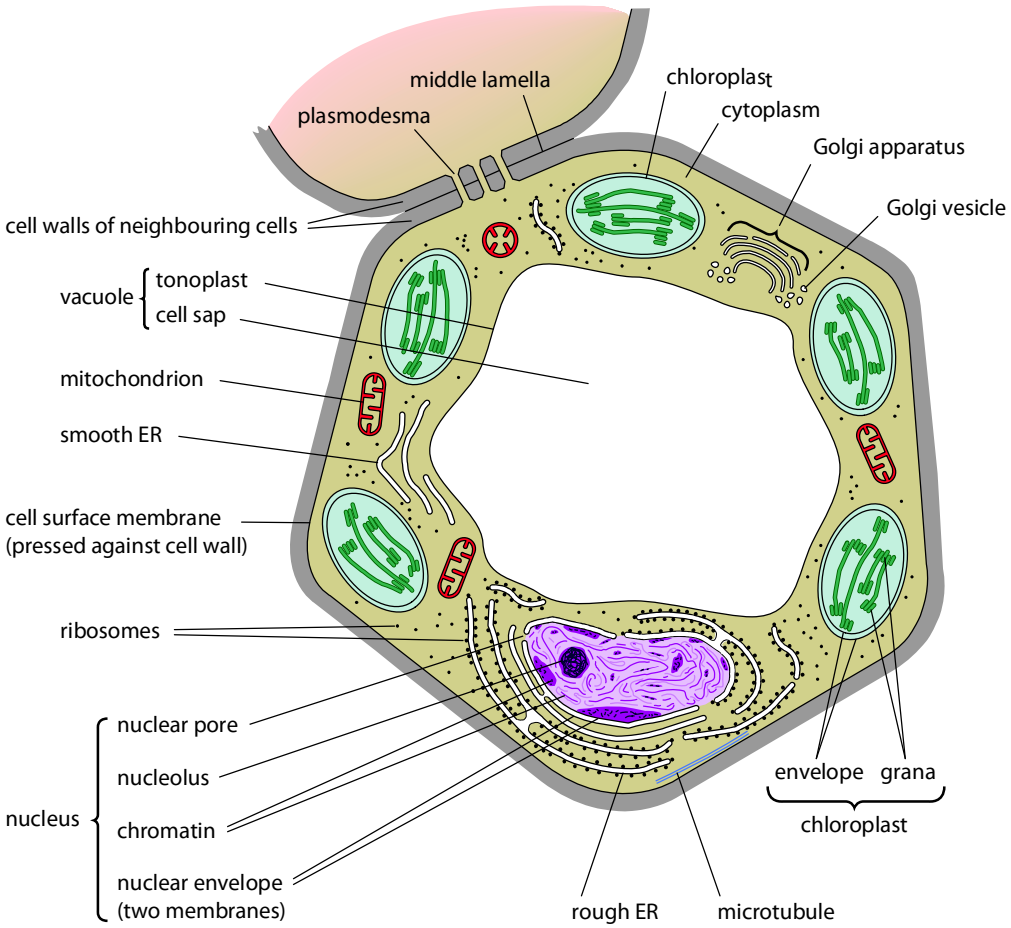


Figure 1.21: Ultrastructure of a typical plant cell as seen with the electron microscope. This drawing is based on many micrographs of plant cells. In reality, the ER is more extensive than shown. Free ribosomes may also be more extensive.

Cell surface membrane

The cell surface membrane is extremely thin (about 7 nm). However, at very high magnifications it can be seen to have three layers – two dark (heavily stained) layers surrounding a narrow, pale interior (Figure 1.22). The membrane is partially permeable and controls exchange between the cell and its environment. Membrane structure is discussed further in Chapter 4.

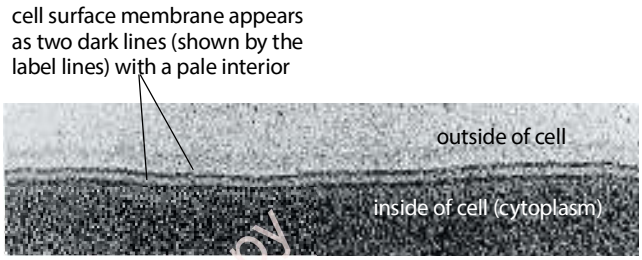


Figure 1.22: Cell surface membrane (×250 000). At this magnification the membrane appears as two dark lines at the edge of the cell.

Microvilli

Microvilli (singular: **microvillus**) are finger-like extensions of the cell surface membrane. They are typical of certain animal cells, such as epithelial cells. Epithelial cells cover the surfaces of structures. The microvilli greatly increase the surface area of the cell surface membrane, as shown in Figure 1.19. This is useful, for example, for reabsorption in the proximal convoluted tubules of the kidney and for absorption of digested food into cells lining the gut.

KEY WORDS

microvilli (singular: **microvillus**): small, finger-like extensions of a cell which increase the surface area of the cell for more efficient absorption or secretion

Question

8 a Using the magnification given, determine the actual maximum diameter of the nucleus shown in Figure 1.23.

b The diameter you have calculated for the nucleus shown in Figure 1.23 is not necessarily the maximum diameter of this nucleus. Explain why this is the case.

IMPORTANT

Use modelling clay to make a spherical shape (a ball), like a nucleus. Try cutting it into two at different places and looking at the sizes of the cut surfaces. This represents the process of sectioning material for examination using a microscope.

Nucleus

The nucleus (Figure 1.23) is the largest cell organelle.

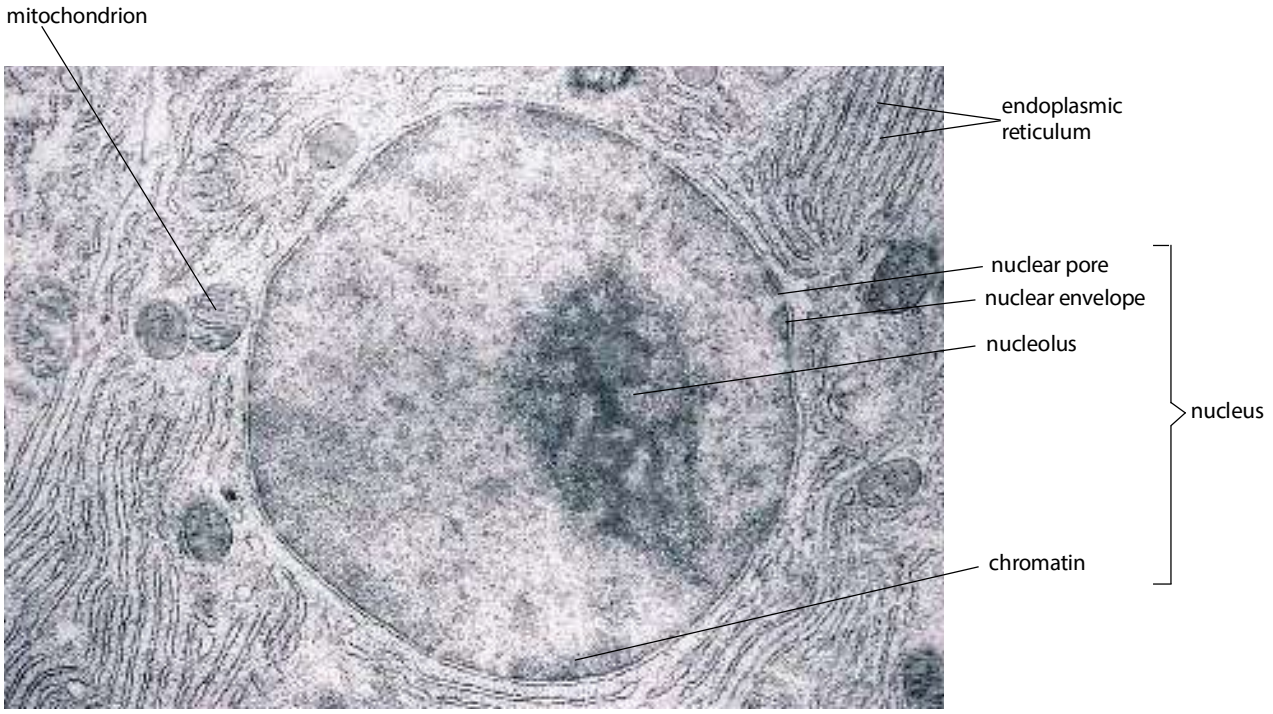


Figure 1.23: Transmission electron micrograph (TEM) of a nucleus. This is the nucleus of a cell from the pancreas of a bat (×11000). The circular nucleus is surrounded by a double-layered nuclear envelope containing nuclear pores. The nucleolus is more darkly stained. Rough ER is visible in the surrounding cytoplasm.

The nuclear envelope

The nucleus is surrounded by two membranes, forming the **nuclear envelope**. The outer membrane of the nuclear envelope is continuous with the endoplasmic reticulum (Figures 1.19 and 1.21).

The nuclear envelope has many small pores called **nuclear pores**. These allow and control exchange between the nucleus and the cytoplasm. Examples of substances leaving the nucleus through the pores are messenger RNA (mRNA), transfer RNA (tRNA) and ribosomes for protein synthesis. Examples of substances entering through the nuclear pores are proteins (to help make ribosomes), nucleotides, ATP (adenosine triphosphate) and some hormones such as thyroid hormone T3.

Chromosomes and chromatin

The nucleus contains the chromosomes. Chromosomes contain DNA, the genetic material. DNA is organised into functional units called genes. Genes control the activities of the cell and inheritance; thus the nucleus controls the cell's activities.

The DNA molecules are so long (a human cell contains about two metres of DNA) that they have to be folded up into a more compact shape to prevent the strands becoming tangled. This is achieved by combining with proteins, particularly with proteins known as histones. The combination of DNA and proteins is known as chromatin. Chromatin also contains some RNA. Thus, chromosomes are made of chromatin (Chapter 5, Section 5.2, Chromosomes).

When a cell is about to divide, the nucleus divides first so that each new cell will have its own nucleus (Chapters 5 and 16).

Also within the nucleusis a structure called the nucleolus.

Nucleolus

The nucleolus appears as a darkly stained, rounded structure in the nucleus (Figure 1.23). As mentioned earlier, one or more may be present, although one is most common. Its function is to make ribosomes using the information in its own DNA. It contains a core of DNA from one or more chromosomes which contain the genes that code for ribosomal RNA (rRNA), the form of RNA used in the manufacture of ribosomes. It also contains genes for making tRNA. Around the core are less dense regions where the ribosomal subunits are assembled, combining the rRNA with ribosomal proteins imported from the cytoplasm. The more ribosomes a cell makes, the larger its nucleolus.

The different parts of the nucleolus only come together during the manufacture of ribosomes. They separate when, as during nuclear division, ribosome synthesis ceases. The nucleolus as a structure then disappears.

Endoplasmic reticulum

When cells were first seen with the electron microscope, biologists were amazed to see so much detailed structure. The existence of much of this had not been suspected. This was particularly true of the **endoplasmic reticulum** (**ER**) (Figures 1.23, 1.24 and 1.28). The membranes of the ER form flattened compartments called sacs or

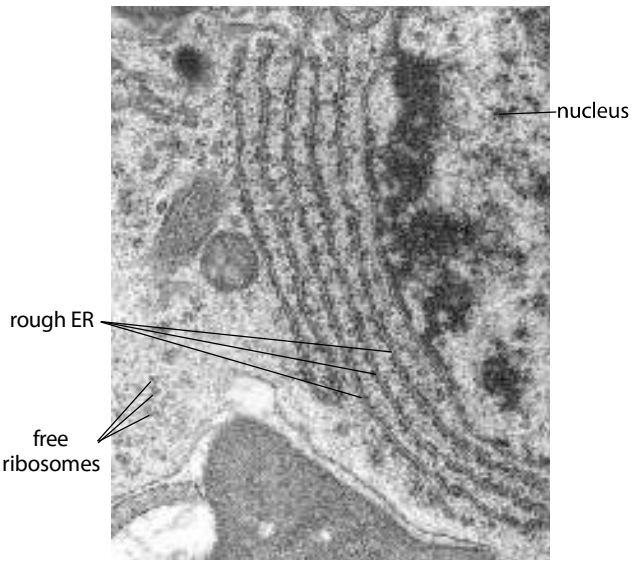


Figure 1.24: TEM of rough ER covered with ribosomes (black dots) (×17 000). Some free ribosomes can also be seen in the cytoplasm on the left.

KEY WORDS

nuclear envelope: the two membranes, situated close together, that surround the nucleus; the envelope is perforated with nuclear pores

nuclear pores: pores found in the nuclear envelope which control the exchange of materials, e.g. mRNA, between the nucleus and the cytoplasm

endoplasmic reticulum (ER): a network of flattened sacs running through the cytoplasm of eukaryotic cells; molecules, particularly proteins, can be transported through the cell inside the sacs separate from the rest of the cytoplasm; ER is continuous with the outer membrane of the nuclear envelope

cisternae. Processes can take place inside the cisternae separated from the cytoplasm. Molecules, particularly proteins, can be transported through the ER separate from the rest of the cytoplasm. The ER is continuous with the outer membrane of the nuclear envelope (Figures 1.19 and 1.21).

Rough endoplasmic reticulum

There are two types of ER: rough ER (RER) and smooth ER (SER). RER is so called because it is covered with many tiny organelles called ribosomes (described later). These are just visible as black dots in Figures 1.23 and 1.24. Ribosomes are the sites of protein synthesis (Chapter 6). They can be found free in the cytoplasm as well as on the RER.

Smooth endoplasmic reticulum

SER has a smooth appearance because it lacks ribosomes. It has a completely different function to RER. It makes lipids and steroids, such as cholesterol and the reproductive hormones oestrogen and testosterone. SER is also a major storage site for calcium ions. This explains why it is abundant in muscle cells, where calcium ions are involved in muscle contraction (Chapter 15, Section 15.3, Muscle contraction). In the liver, SER is involved in drug metabolism.

Ribosomes

Ribosomes are very small and are not visible with a light microscope. At very high magnifications using an electron microscope they can be seen to consist of two subunits: a large and a small subunit. The sizes of structures this small are often quoted in S units (Svedberg units). S units are a measure of how rapidly substances sediment in a high speed centrifuge (an ultracentrifuge). The faster they sediment, the higher the S number. Eukaryotic ribosomes are 80S ribosomes. The ribosomes of prokaryotes are 70S ribosomes, so are slightly smaller. Mitochondria and chloroplasts contain 70S ribosomes, revealing their prokaryotic origins (see the sections on mitochondria and chloroplasts).

Ribosomes are made of roughly equal amounts by mass of ribosomal RNA (rRNA) and protein. Their three-dimensional structure has now been worked out (Figure 1.25). Ribosomes allow all the interacting molecules involved in protein synthesis, such as mRNA, tRNA, amino acids and regulatory proteins, to gather together in one place (Chapter 6, Section 6.5, Protein synthesis).

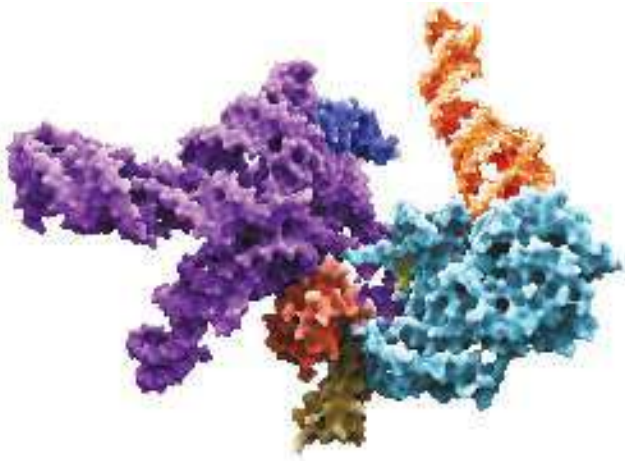


Figure 1.25: Structure of the human 80S ribosome.

Golgi apparatus

The **Golgi apparatus** is a stack of flattened sacs called cisternae (Figure 1.26). More than one Golgi apparatus may be present in a cell. The stack is constantly being formed at one end from vesicles which bud off from the ER, and are broken down again at the other end to form **Golgi vesicles**. The stack of sacs together with the associated vesicles is referred to as the Golgi apparatus or Golgi complex.

KEY WORDS

ribosome: a tiny organelle found in large numbers in all cells; prokaryotic ribosomes are about 20 nm in diameter while eukaryotic ribosomes are about 25 nm in diameter

Golgi apparatus (Golgi body, Golgi complex): an organelle found in eukaryotic cells; the Golgi apparatus consists of a stack of flattened sacs, constantly forming at one end and breaking up into Golgi vesicles at the other end

Golgi vesicles: carry their contents to other parts of the cell, often to the cell surface membrane for secretion; the Golgi apparatus chemically modifies the molecules it transports, e.g. sugars may be added to proteins to make glycoproteins

The Golgi apparatus collects and processes molecules, particularly proteins from the RER. It contains hundreds of enzymes for this purpose. After processing, the molecules can be transported in Golgi vesicles to other parts of the cell or out of the cell. Releasing molecules from the cell is called secretion and the pathway followed by the molecules is called the secretory pathway. These are some examples of the functions of the Golgi apparatus

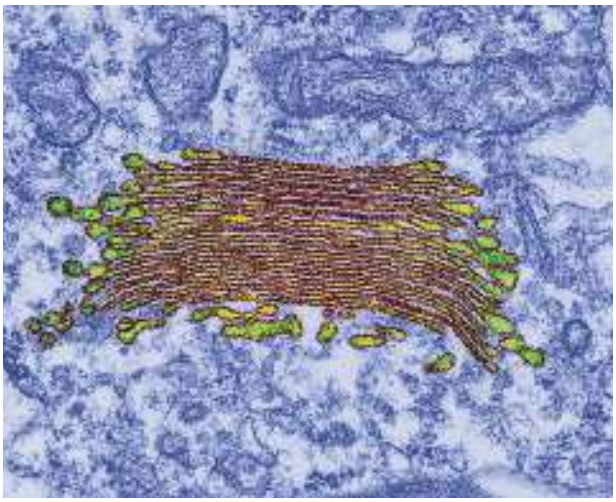


Figure 1.26: TEM of a Golgi apparatus. A central stack of saucer-shaped sacs can be seen budding off small Golgi vesicles (green). These may form secretory vesicles whose contents can be released at the cell surface by exocytosis (Chapter 4).

- Golgi vesicles are used to make lysosomes.
- Sugars are added to proteins to make molecules known as glycoproteins.
- Sugars are added to lipids to make glycolipids. Glycoproteins and glycolipids are important components of membranes (Chapter 4, Section 4.2, Structure of membranes) and are important molecules in cell signalling (Chapter 4, Section 4.4, Cell signalling).
- During plant cell division, Golgi enzymes are involved in the synthesis of new cell walls.
- In the gut and the gas exchange system, cells called goblet cells release a substance called mucin from the Golgi apparatus (Chapter 9, Section 9.4, Warming and cleaning the air). Mucin is one of the main components of mucus.

Lysosomes

Lysosomes are simple sacs, surrounded by a single membrane. In animal cells they are usually 0.1–0.5 μm in diameter (Figure 1.27). In plant cells the large central vacuole may act as a lysosome, although lysosomes similar to those in animal cells are also seen in the cytoplasm.

KEY WORD

lysosome: a spherical organelle found in eukaryotic cells; it contains digestive (hydrolytic) enzymes and has a variety of destructive functions, such as removal of old cell organelles

Figure 1.27: Lysosomes (orange) in a mouse kidney cell (×55 000). They contain cell structures in the process of digestion. Cytoplasm is coloured blue here.

Lysosomes contain digestive enzymes. The enzymes are called hydrolases because they carry out hydrolysis reactions. These enzymes must be kept separate from the rest of the cell to prevent damage. Lysosomes are responsible for the breakdown (digestion) of unwanted substances and structures such as old organelles or even whole cells. Hydrolysis works fastest in an acid environment so the contents of lysosomes are acidic, pH 4–5 compared with 6.5–7.0 in the surrounding cytoplasm. Among the 60+ enzymes contained in lysosomes are proteases, lipases and nucleases which break down proteins, lipids and nucleic acids respectively. The enzymes are synthesised on RER and delivered to lysosomes via the Golgi apparatus.

The activities of lysosomes can be split into the four categories discussed below.

Getting rid of unwanted cell components

Lysosomes can engulf and destroy unwanted cell components, such as molecules or organelles, that are located inside the cell.

Endocytosis

Endocytosis is described in more detail in Chapter 4 (Section 4.5, Movement of substances across membranes). Material may be taken into the cell by endocytosis, for example when white blood cells engulf bacteria. Lysosomes may fuse with the endocytic vacuoles formed and release their enzymes to digest the contents.

Exocytosis

Lysosomal enzymes may be released from the cell for extracellular digestion. An example is the replacement of cartilage by bone during development. The heads of sperms contain a special lysosome, the acrosome, for digesting a path through the layers of cells surrounding the egg just before fertilisation.

Self-digestion

The contents of lysosomes are sometimes released into the cytoplasm. This results in the whole cell being digested (a process called autolysis). This may be part of normal development, as when a tadpole tail is reabsorbed during metamorphosis or when a uterus is restored to its normal size after pregnancy. It also occurs after the death of an individual as membranes lose their partial permeability.

Mitochondria

Structure

The structure of the mitochondrion (plural: mitochondria) as seen with the electron microscope is visible in Figures 1.18, 1.28 and 12.10. Mitochondria are usually about 1 μm in diameter and can be various shapes, often sausage-shaped as in Figure 1.28. They are surrounded by two membranes (an envelope). The inner membrane is folded to form finger-like **cristae** (singular: **crista**) which project into the interior of the mitochondrion which is called the matrix. The space between the two membranes is called the intermembrane space.

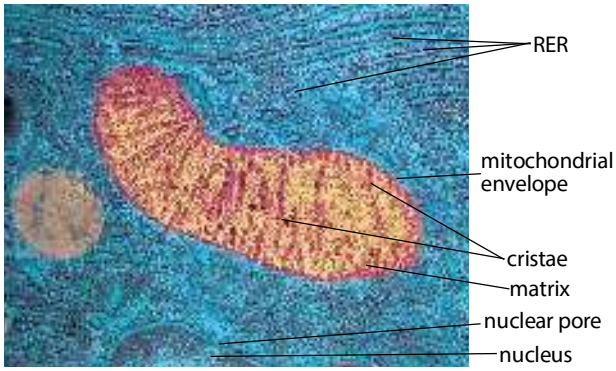


Figure 1.28: Mitochondrion (orange) with its double membrane (envelope); the inner membrane is folded to form cristae (×20 000). Mitochondria are the sites of aerobic cell respiration. Note also the RER.

The number of mitochondria in a cell is very variable. As they are responsible for aerobic respiration, it is not surprising that cells with a high demand for energy, such as liver and muscle cells, contain large numbers of mitochondria. A liver cell may contain as many as 2000 mitochondria. If you exercise regularly, your muscles will make more mitochondria.

Functions of mitochondria and the role of ATP

The main function of mitochondria is to carry out aerobic respiration, although they do have other functions, such as the synthesis of lipids. During respiration, a series of reactions takes place in which energy is released from energy-rich molecules such as sugars and fats. Most of the energy is transferred to molecules of **ATP** (**adenosine triphosphate**). This is the energy-carrying molecule found in all living cells. It is known as the universal energy carrier.

KEY WORDS

cristae (singular: **crista**): folds of the inner membrane of the mitochondrial envelope on which are found stalked particles of ATP synthase and electron transport chains associated with aerobic respiration

ATP (**adenosine triphosphate**): the molecule that is the universal energy currency in all living cells; the purpose of respiration is to make ATP

The reactions of respiration take place in solution in the matrix and in the inner membrane (cristae). The matrix contains enzymes in solution, including those of the Krebs cycle. Electron carriers are found in the cristae. For more detail, see Chapter 12 (Section 12.2).

Once made, ATP leaves the mitochondrion and, as it is a small, soluble molecule, it can spread rapidly to all parts of the cell where energy is needed. Its energy is released by breaking the molecule down to **ADP** (**adenosine diphosphate**). This is a hydrolysis reaction. The ADP can then be recycled in a mitochondrion for conversion back to ATP during aerobic respiration.

The endosymbiont theory

Note: The endosymbiont theory is extension content, and is not part of the syllabus.

In the 1960s, it was discovered that mitochondria and chloroplasts contain ribosomes which are slightly smaller than those in the cytoplasm and are the same size as those found in bacteria. Cytoplasmic ribosomes are 80S, while those of bacteria, mitochondria and chloroplasts are 70S. It was also discovered in the 1960s that mitochondria and chloroplasts contain small, circular DNA molecules, also like those found in bacteria. It was later proved that mitochondria and chloroplasts are, in effect, ancient bacteria which now live inside the larger cells of animals and plants (see 'Thinking outside the box' at the beginning of this chapter). This is known as the endosymbiont theory. 'Endo' means 'inside' and a 'symbiont' is an organism which lives in a mutually beneficial relationship with another organism. The DNA and ribosomes of mitochondria and chloroplasts are still active and responsible for the coding and synthesis of certain vital proteins, but mitochondria and chloroplasts can no longer live independently. Mitochondrial ribosomes are just visible as tiny dark orange dots in the mitochondrial matrix in Figure 1.28.

Microtubules and microtubule organising centres (MTOCs)

Microtubules are long, rigid, hollow tubes found in the cytoplasm. They are very small, about 25 nm in diameter. Together with actin filaments and intermediate filaments (not discussed in this book), they make up the cytoskeleton, an essential structural component of cells which helps to determine cell shape.

Microtubules are made of a protein called tubulin. Tubulin has two forms, α-tubulin (alpha-tubulin) and β-tubulin (beta-tubulin). α- and β-tubulin molecules combine to form dimers (double molecules). These dimers are then joined end to end to form long 'protofilaments'. This is an example of polymerisation, the process by which giant molecules are made by joining together many identical subunits. Thirteen protofilaments line up alongside each other in a ring to form a cylinder with a hollow centre. This cylinder is the microtubule. Figure 1.29a shows the helical pattern formed by neighbouring α- and β-tubulin molecules.

Apart from their mechanical function of support, microtubules have a number of other functions.

- Secretory vesicles and other organelles and cell components can be moved along the outside surfaces of the microtubules, forming an intracellular transport system, as in the movement of Golgi vesicles during exocytosis.
- During nuclear division (Chapter 5), a spindle made of microtubules is used for the separation of chromatids or chromosomes.
- Microtubules form part of the structure of centrioles.
- Microtubules form an essential part of the mechanism involved in the beating movements of cilia and flagella.

The assembly of microtubules from tubulin molecules is controlled by special locations in cells called microtubule organising centres (MTOCs). These are discussed further in the following section on centrioles. Because of their simple construction, microtubules can be formed and broken down very easily at the MTOCs, according to need.

KEY WORDS

ADP (**adenosine diphosphate**): the molecule that is converted to ATP by addition of phosphate (a reaction known as phosphorylation) during cell respiration; the enzyme responsible is ATP synthase; the reaction requires energy

microtubules: tiny tubes made of a protein called tubulin and found in most eukaryotic cells; microtubules have a large variety of functions, including cell support and determining cell shape; the 'spindle' on which chromatids and chromosomes separate during nuclear division is made of microtubules

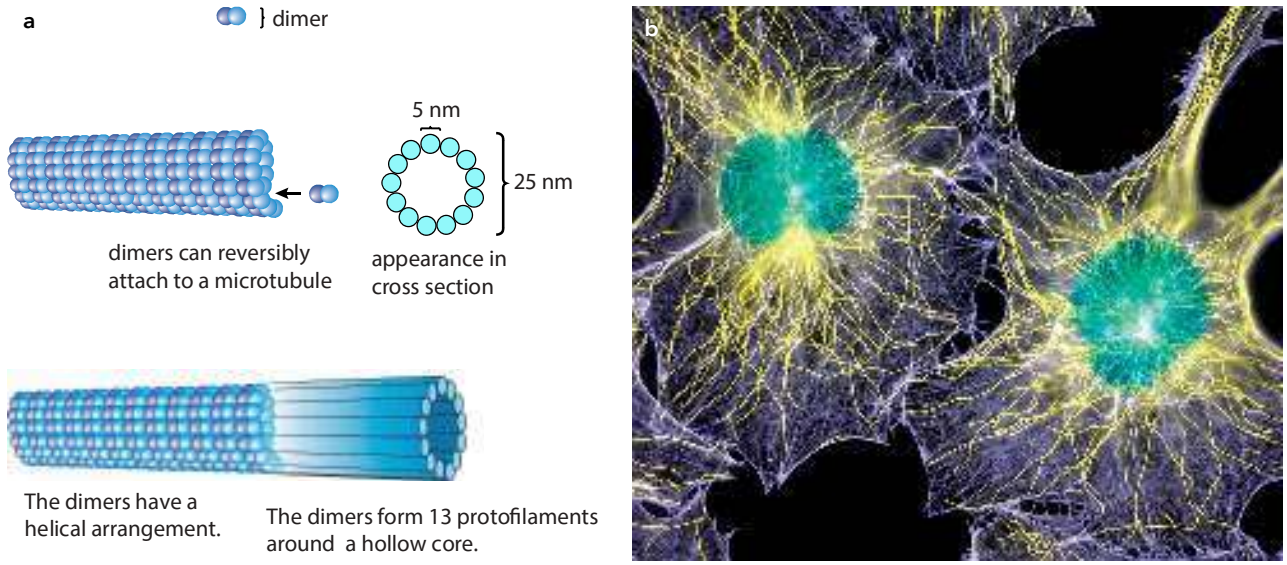


Figure 1.29: a The structure of a microtubule and **b** the arrangement of microtubules in two cells. The microtubules are coloured yellow.

Centrioles and centrosomes

Note: Centrosomes are extension content, and are not part of the syllabus.

The extra resolution of the electron microscope reveals that just outside the nucleus of animal cells there are really *two* **centrioles** and not one as it appears under the light microscope (compare Figures 1.4 and 1.19).

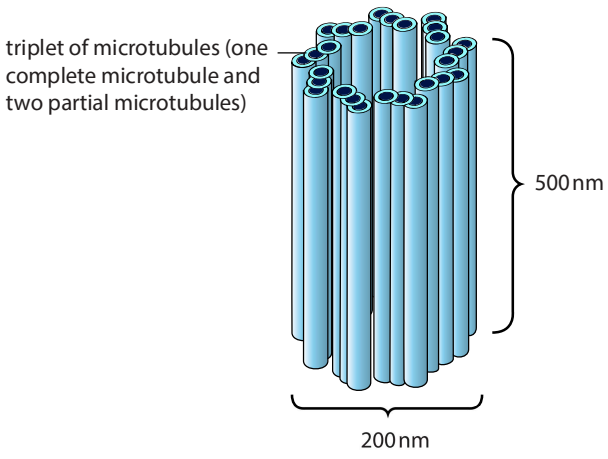


Figure 1.30: The structure of a centriole. It consists of nine groups of microtubules. Each group is made up of three microtubules, a triplet.

They lie close together and at right angles to each other in a region known as the **centrosome**. Centrioles and the centrosome are absent from most plant cells.

A centriole is a hollow cylinder about 500 nm long, formed from a ring of short microtubules. Each centriole contains nine triplets of microtubules (Figures 1.30 and 1.31).

Until recently, it was believed that centrioles acted as MTOCs for the assembly of the microtubules that make up the spindle during nuclear division (Chapter 5). It is now known that this is done by the centrosome, but does not involve the centrioles. However, centrioles are needed for the production of cilia. Centrioles are found at the bases of cilia and flagella, where they are known as basal bodies. The centrioles act as MTOCs. The microtubules that extend from the basal bodies into the cilia and flagella are essential for the beating movements of these organelles.

KEY WORDS

centriole: one of two small, cylindrical structures, made from microtubules, found just outside the nucleus in animal cells, in a region known as the centrosome; they are also found at the bases of cilia and flagella

centrosome: the main microtubule organising centre (MTOC) in animal cells

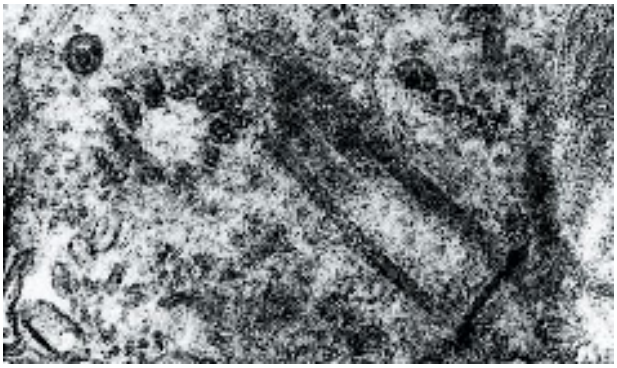


Figure 1.31: Centrioles in transverse and longitudinal section (TS and LS) (×86 000). The one on the left is seen in TS and clearly shows the nine triplets of microtubules which make up the structure.

KEY WORDS

cilia (singular: **cilium**): whip-like structures projecting from the surface of many animal cells and the cells of many unicellular organisms; they beat, causing locomotion or the movement of fluid across the cell surface

flagella (singular: **flagellum**): whip-like structures projecting from the surface of some animal cells and the cells of many unicellular organisms; they beat, causing locomotion or the movement of fluid across the cell surface; they are identical in structure to cilia, but longer

Note: the structure of flagella is extension content, and not part of the syllabus.

Cilia and flagella

Cilia (singular: **cilium**) and **flagella** (singular: **flagellum**) have identical structures. They are whip-like, beating extensions of many eukaryotic cells. Each is surrounded by an extension of the cell surface membrane. They were given different names before their structures were discovered: flagella are long and found usually one or two per cell, whereas cilia are short and often numerous.

Structure

Cilia and flagella are extremely complicated structures, composed of over 600 different polypeptides. This complexity results in very fine control of how they beat.

The structure of a cilium is shown in Figure 1.32. Cilia have two central microtubules and a ring of nine microtubule doublets (MTDs) around the outside. This is referred to as a '9 + 2' structure. Each MTD contains an A and a B microtubule (Figure 1.32a). The wall of the A microtubule is a complete ring of 13 protofilaments and the B microtubule attached is an incomplete ring with only 10 protofilaments (see Figure 1.32a). Figure 1.32a shows that each A microtubule has inner and outer arms. These are made of the protein dynein. They connect with the B microtubules of neighbouring MTDs during beating. If you imagine the microtubule in three dimensions, there are two rows of several hundred dynein arms along the outside of each A microtubule. The whole cylindrical structure inside the cell surface membrane is called the axoneme.

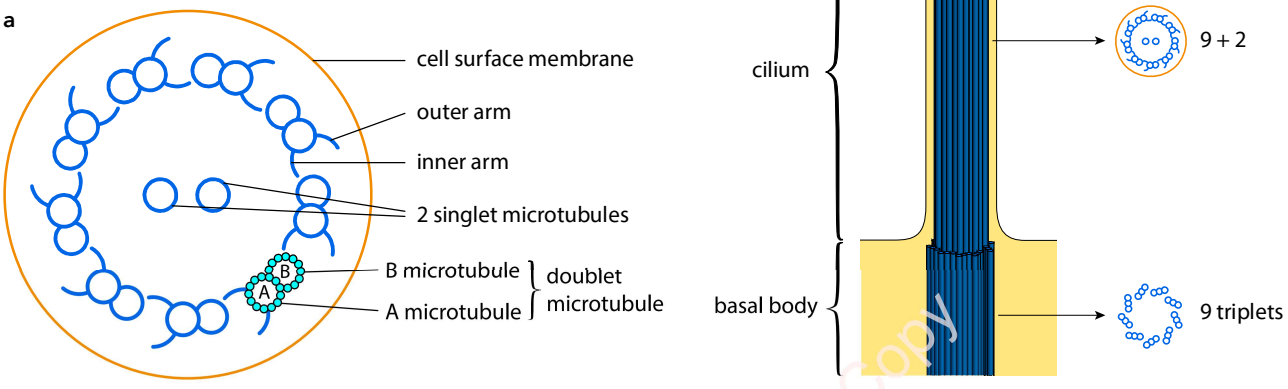


Figure 1.32: The structure of a cilium. **a** A cilium seen in TS. Note the '9 + 2' arrangement of microtubules. **b** A cilium. TSs of the cilium (9 + 2) and basal body (9 triplets) are also shown.

At the base of each cilium and flagellum is a structure called the basal body which is identical in structure to the centriole. We now know that centrioles replicate themselves to produce these basal bodies, and that cilia and flagella grow from basal bodies. Figure 1.33 is a scanning electron micrograph of cilia in the respiratory tract.

Figure 1.33: Scanning electron micrograph of cilia in the respiratory tract

Beating mechanism

The beating motion of cilia and flagella is caused by the dynein (protein) arms making contact with, and moving along, neighbouring microtubules. This produces the force needed for cilia to beat. As neighbouring MTDs slide past each other, the sliding motion is converted into bending by other parts of the cilium.

Functions

If the cell is attached to something so that it cannot move, fluid will move past the cell. If the cell is not attached, the cell will swim through the fluid. Single-celled organisms can therefore use the action of cilia and flagella for locomotion. You will easily be able to find videos of such motion on the internet. In vertebrates, beating cilia are found on some epithelial cells, such as those lining the airways (Chapter 9). Here more than 10 million cilia may be found per mm^2. They maintain a flow of mucus which removes debris such as dust and bacteria from the respiratory tract.

Question

9 In vertebrates, beating cilia are also found on the epithelial cells of the oviduct (the tube connecting the ovary to the uterus). Suggest what function cilia have in the oviduct.

Chloroplasts

The structure of the chloroplast as seen with the electron microscope is shown in Figures 1.20, 1.21 and 1.34. You can also see a higher-resolution micrograph in Figure 13.4. Chloroplasts tend to have an elongated shape and a diameter of about 3–10 μm (compare 1 μm diameter for mitochondria). Like mitochondria, they are surrounded by two membranes, which form the chloroplast envelope.

The main function of chloroplasts is to carry out photosynthesis. During the first stage of photosynthesis (the light-dependent stage), light energy is absorbed by photosynthetic pigments, particularly chlorophyll. The pigments are found on the membranes of the chloroplast.

The membrane system consists of fluid-filled sacs called **thylakoids**, which spread out like sheets in three dimensions. In places, the thylakoids form flat, disc-like structures that stack up like piles of coins, forming structures called grana (from their appearance in the light microscope; 'grana' means grains).

KEY WORD

thylakoid: a flattened, membrane-bound, fluid-filled sac which is the site of the light-dependent reactions of photosynthesis in a chloroplast

The second stage of photosynthesis (the light-independent stage) uses the energy and reducing power generated during the first stage to convert carbon dioxide into sugars. This takes place in the stroma. The sugars made may be stored in the form of starch grains in the stroma (Figures 1.20 and 13.3 and 13.4).

Lipid droplets are also seen in the stroma. They appear as black spheres in electron micrographs (Figure 1.34). They are reserves of lipid for making membranes or are formed from the breakdown of internal membranes as the chloroplast ages.

Like mitochondria, chloroplasts have their own protein synthesising machinery, including 70S ribosomes and

circular DNA. In electron micrographs, the ribosomes can just be seen as small black dots in the stroma (Figure 13.4).

As with mitochondria, it has been shown that chloroplasts originated as endosymbiotic bacteria, in this case photosynthetic blue-green bacteria. The endosymbiont theory is discussed in more detail in the earlier section on mitochondria.

Cell walls

Structure

The first walls formed by plant cells are known as primary walls. They are relatively rigid. The primary wall consists of parallel fibres of the polysaccharide cellulose running through a matrix of other polysaccharides such as pectins and hemicelluloses. Cellulose fibres are inelastic and have high tensile strength, meaning they are difficult to break by pulling on each end. This makes it difficult to stretch the wall, for example when water enters the cell by osmosis. The structure of cellulose is described in Chapter 2.

In most cells extra layers of cellulose are added to the first layer of the primary wall, forming a secondary wall. In a given layer the cellulose fibres are parallel, but the fibres of different layers run in different directions forming a cross-ply structure which is stronger as a result (see Figure 2.10).

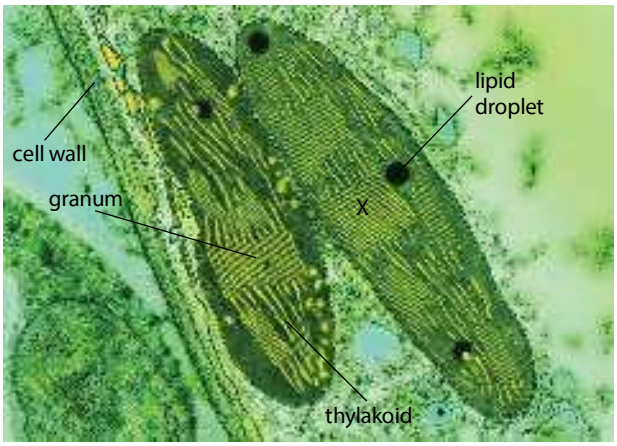


Figure 1.34: Two chloroplasts (×16 000). Thylakoids (yellow) run through the stroma (dark green) and are stacked in places to form grana. Black circles among the thylakoids are lipid droplets. See also Figures 13.3 and 13.4. Chloroplast **X** is referred to in Question 3b.

Some cell walls become even stronger and more rigid by the addition of lignin. Xylem vessel elements and sclerenchyma are examples (Chapter 7). Lignin adds compressional strength to tensile strength (it prevents buckling). It is what gives wood (secondary xylem) its strength and is needed for support in shrubs and trees.

Functions

Some of the main functions of cell walls are summarised below.

- Mechanical strength and support for individual cells and the plant as a whole. Lignification is one means of support. Turgid tissues are another means of support that is dependent on strong cell walls.
- Cell walls prevent cells from bursting by osmosis if cells are surrounded by a solution with a higher water potential (Chapter 2).
- Different orientations of the layers of cellulose fibres help determine the shapes of cells as they grow.
- The system of interconnected cell walls in a plant is called the apoplast. It is a major transport route for water, inorganic ions and other materials (Chapter 7).
- Living connections through neighbouring cell walls, the plasmodesmata, help form another transport pathway through the plant known as the symplast (Chapter 7).
- The cell walls of the root endodermis are impregnated with suberin, a waterproof substance that forms a barrier to the movement of water, thus helping in the control of water and mineral ion uptake by the plant (Chapter 7).
- Epidermal cells often have a waterproof layer of waxy cutin, the cuticle, on their outer walls. This helps reduce water loss by evaporation.

Vacuoles

As we have seen, animal cell vacuoles are relatively small and include phagocytic vacuoles, food vacuoles and autophagic vacuoles.

Unlike animal cells, plant cells typically have a large central vacuole (Figure 1.20). Some examples of the functions of the large central vacuole of plants are listed below. It is useful to try to remember one or two of these examples.

Support

The solution in the vacuole is relatively concentrated. Water therefore enters the vacuole by osmosis, inflating the vacuole and causing a build-up of pressure. A fully inflated cell is described as turgid. Turgid tissues help to support the stems of plants that lack wood (wilting demonstrates the importance of this).

Lysosomal activity

Plant vacuoles may contain hydrolases and act as lysosomes.

Secondary metabolites

Plants contain a wide range of chemicals known as secondary metabolites which, although not essential for growth and development, contribute to survival in various ways. These are often stored in vacuoles. Examples of their functions are:

- Anthocyanins are pigments that are responsible for most of the red, purple, pink and blue colours of flowers and fruits. They attract pollinators and seed dispersers.
- Certain alkaloids and tannins deter herbivores from eating the plant.
- Latex, a milky fluid, can accumulate in vacuoles, for example in rubber trees. The latex of the opium poppy contains alkaloids such as morphine from which opium and heroin are obtained.

Food reserves

Food reserves, such as sucrose in sugar beet, or mineral salts, may be stored in the vacuole. Protein-storing vacuoles are common in seeds.

Waste products

Waste products, such as crystals of calcium oxalate, may be stored in vacuoles.

Growth in size

Osmotic uptake of water into the vacuole is responsible for most of the increase in volume of plant cells during growth. The vacuole occupies up to a third of the total cell volume.

PRACTICAL ACTIVITY 1.3

Work in groups of ten. Each group should make one copy of the following table on stiff card.

START	Photosynthesis occurs in this organelle
Chloroplast	Chromosomes are found in this structure in eukaryotic cells
Nucleus	These are found on rough endoplasmic reticulum (RER)
Ribosomes	This structure contains cellulose as a strengthening material
Cell wall	Makes ribosomes
Nucleolus	Site of ATP synthesis in aerobic respiration
Mitochondrion	Makes lysosomes
Golgi apparatus	Has a '9 + 2' arrangement of microtubules
Cilium	Mainly contains digestive enzymes
Lysosome	END

Cut up the card so that each piece of card has one term and one description (one row of the table). There are therefore ten cards.

Shuffle the cards and take one each. The student with the **START** card reads out the description and the student who has the correct matching term reads out **THE** correct term from their card. They then read out the description on their card. This continues until it reaches the **END** card. Your teacher will help if you get stuck.

The cards can be reshuffled and the activity repeated to see if you can do it faster the second time.

1.7 Bacteria

You will recall that there are two fundamental types of cell: prokaryotes and eukaryotes. The plant and animal cells you have studied so far are eukaryotic cells. Bacteria are prokaryotes and their cells are much simpler than those of eukaryotes. Prokaryotic cells are generally about 1000 times smaller in volume and lack a nucleus that is surrounded by a double membrane. Prokaryotes are thought to have been the first living organisms on Earth. The earliest known fossil prokaryotes are about 3.5 billion years old (the Earth was formed about 4.5 billion years ago). Most biologists believe that eukaryotes evolved from prokaryotes about 2 billion years ago. There are two groups of prokaryotes, known as Bacteria and Archaea. (The classification of living organisms is discussed in Chapter 18.) We consider only Bacteria in this book.

Structure of bacteria

Figure 1.35 shows the structure of a typical **bacterium** (plural: **bacteria**). The left side of the diagram shows the structures that are always present. The right side of the diagram shows the structures which are sometimes found in bacteria.

KEY WORD

bacteria (singular: **bacterium**): a group of single-celled prokaryotic microorganisms; they have a number of characteristics, such as the ability to form spores, which distinguish them from the other group of prokaryotes known as Archaea

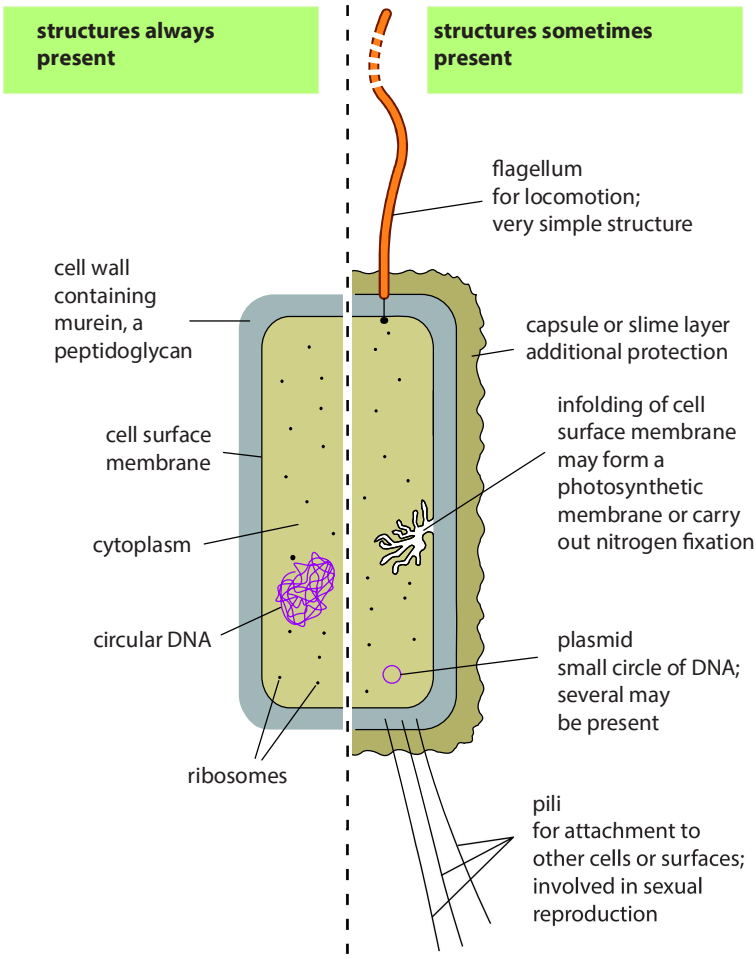


Figure 1.35: Diagram of a bacterium. Cells are generally about 1–5 μm in diameter.

Cell wall

Bacterial cell walls contain a strengthening material called **peptidoglycan**. The cell wall protects the bacterium and is essential for its survival. It prevents the cell from swelling up and bursting if water enters the cell by osmosis.

KEY WORD

peptidoglycan: a polysaccharide combined with amino acids; it is also known as murein; it makes the bacterial cell wall more rigid

Cell surface membrane

Like all cells, bacterial cells are surrounded by a cell surface membrane.

Cytoplasm

The cytoplasm does not contain any double membrane-bound organelles (such as mitochondria).

Circular DNA

The DNA molecule in bacteria is circular. It is found in a region called the nucleoid, which also contains proteins and small amounts of RNA. It is not surrounded by a double membrane, unlike the nucleus of eukaryotes. There may be more than one copy of the DNA molecule in a given cell.

Ribosomes

Bacterial ribosomes are 70S ribosomes, slightly smaller than the 80S ribosomes of eukaryotes.

Flagellum

Some bacteria are able to swim because they have one or more flagella. Bacterial flagella have a much simpler structure than eukaryotic flagella. The bacterial flagellum is a simple hollow cylinder made of identical protein molecules. It is a rigid structure, so it does not bend, unlike the flagella in eukaryotes. It is wave-shaped and works by rotating at its base like a propeller to push the bacterium through its liquid environment. As a result, the bacterium moves forward with a corkscrew-shaped motion.

Infolding of cell surface membrane

In some bacteria, the cell surface membrane folds into the cell forming an extra surface on which certain biochemical reactions can take place. In blue–green bacteria, for example, the infolded membrane contains photosynthetic pigments which allow photosynthesis to take place. In some bacteria, nitrogen fixation takes place on the infolded membrane. Nitrogen fixation is the ability to convert nitrogen in the air to nitrogen-containing compounds, such as ammonia, inside the cell. All life depends on nitrogen fixation. Eukaryotes cannot carry out nitrogen fixation.

Capsule

Some bacteria are surrounded by an extra layer outside the cell wall. This may take the form of a capsule or a slime layer. A capsule is a definite structure, made mostly of polysaccharides. A slime layer is more diffuse and is easily washed off. Both help to protect the bacterium from drying out and may have other protective functions. For example, a capsule helps protect some bacteria from antibiotics. Some capsules prevent white blood cells known as phagocytes from engulfing disease-causing bacteria.

Plasmid

A **plasmid** is a small circle of DNA separate from the main DNA of the cell. It contains only a few genes. Many plasmids may be present in a given cell. The genes have various useful functions. Commonly, plasmids contain genes that give resistance to particular antibiotics, such as penicillin. Plasmids can copy themselves independently of the chromosomal DNA and can spread rapidly from one bacterium to another. Plasmid DNA is not associated with protein and is referred to as 'naked' DNA.

KEY WORD

plasmid: a small circular piece of DNA in a bacterium (not its main chromosome); plasmids often contain genes that provide resistance to antibiotics

Pili (singular: pilus)

Pili are fine protein rods. They vary in length and stiffness. One to several hundred may be present on the outside of the cell. They are used for attachment and interactions with other cells or surfaces. They allow the transfer of genes, including plasmids, from one bacterium to another during conjugation.

1.8 Comparing prokaryotic cells with eukaryotic cells

Table 1.3 compares prokaryotic cells with eukaryotic cells.

Prokaryotes	Eukaryotes
Prokaryotes are thought to have evolved about 3.5 billion years ago.	Eukaryotes are thought to have evolved about 1.5 billion years ago.
Their typical diameter is 1–5 μm.	Cells are up to 40 μm diameter and up to 1000 times the volume of prokaryotic cells.
DNA is circular and free in the cytoplasm; it is not surrounded by a double membrane.	DNA is not circular and is contained in a nucleus. The nucleus is surrounded by a double membrane (the nuclear envelope).
70S ribosomes are present (smaller than those of eukaryotes).	80S ribosomes are present (larger than those of prokaryotes).
Very few types of cell organelle are present. No separate membrane-bound organelles are present.	Many types of cell organelle are present. • Some organelles are surrounded by a single membrane (e.g. lysosomes, Golgi apparatus, vacuoles, ER). • Some are surrounded by an envelope of two membranes (e.g. nucleus, mitochondrion, chloroplast). • Some have no membrane (e.g. ribosomes, centrioles, microtubules).
The cell wall contains peptidoglycan (a polysaccharide combined with amino acids).	A cell wall is sometimes present (e.g. in plants and fungi); it contains cellulose or lignin in plants, and chitin (a nitrogen-containing polysaccharide similar to cellulose) in fungi.
Flagella are simple and lack microtubules; they project outside the cell surface membrane so they are extracellular (outside the cell).	Flagella (and cilia) are complex with a '9 + 2' arrangement of microtubules; they are surrounded by the cell surface membrane so they are intracellular (inside the cell).
Cell division occurs by binary fission (the cell splits into two); it does not involve a spindle (see Chapter 6).	Cell division takes place by mitosis or meiosis and involves a spindle (see Chapter 6).
Some carry out nitrogen fixation.	None carries out nitrogen fixation.

Table 1.3: Comparing prokaryotic cells and eukaryotic cells.

Question

10 List the structural features that prokaryotic and eukaryotic cells have in common. Briefly explain why each of the structures you have listed is essential.

1.9 Viruses

In 1852, a Russian scientist discovered that certain diseases could be transmitted by agents that, unlike bacteria, could pass through very fine filters. This was the first evidence for the existence of viruses. **Viruses** are tiny 'particles' which are much smaller than bacteria and are on the boundary between what we think of as living and non-living. Unlike prokaryotes and eukaryotes, viruses do not have a cell structure. In other words, they

KEY WORD

virus: a very small (20–300 nm) infectious particle which can replicate only inside living cells; it consists of a molecule of DNA or RNA (the genome) surrounded by a protein coat; an outer lipid envelope may also be present

are not surrounded by a partially permeable membrane containing cytoplasm with ribosomes. They are much simpler in structure. They consist only of the following:

- a self-replicating molecule of DNA or RNA (the genome or complete genetic instructions)
- a protective coat of protein molecules called a capsid
- (some viruses only) a membrane-like outer layer, called the envelope, that is made of **phospholipids**. (The structure of phospholipids is described in Chapter 2.) Proteins may project from the envelope.

Figure 1.36 shows the structure of a virus with an envelope. Viruses typically have a very symmetrical shape. The protein coat (or capsid) is made up of separate protein molecules, each of which is called a capsomere.

KEY WORD

phospholipid: a lipid to which phosphate is added; the molecule is made up of a glycerol molecule, two fatty acids and a phosphate group; a double layer (a bilayer) of phospholipids forms the basic structure of all cell membranes

Viruses range in size from about 20 nm to 300 nm (about 50 times smaller on average than bacteria).

All viruses are parasitic because they can only reproduce by infecting and taking over living cells. The virus DNA or RNA takes over the protein synthesising machinery of the host cell, which then helps to make new virus particles.

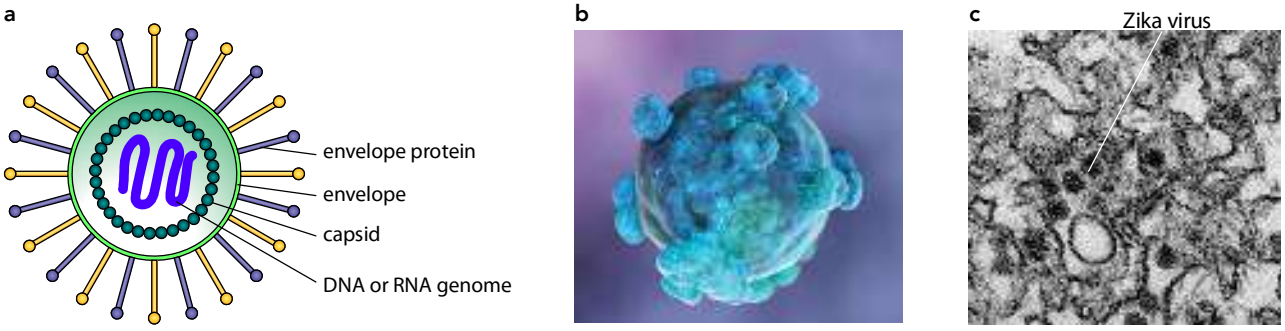


Figure 1.36: a The structure of a virus with an envelope; **b** model of a Zika virus. The virus is an RNA virus. Its capsid has an outer envelope; **c** electron micrograph of a cell infected by Zika virus. The virus particles are the darkly stained roughly spherical structures. Each virus particle is about 40 nm in diameter.

REFLECTION

Think about everything you know about cells. What answers would you give to the following questions?

- What is a cell?
- Why are all living things made of cells?

Look back at the differences between eukaryotic and prokaryotic cells.

- Write down a list of criteria to compare the success of prokaryotic and eukaryotic cells.
- Suggest why trying to compare the success of prokaryotic and eukaryotic cells may be a meaningless exercise. (Tip: think about the meaning of the word 'success'.)

Personal reflection questions

Changing from studying at GCSE to studying at AS Level is a big jump. Has anything surprised you about the change? Are you confident about being able to adapt the way you work? If not, what particular concerns do you have?

You have studied cells in Chapter 1 and learnt a lot about their structure and function. The Reflection activity gives you a chance to use this information to think again about cells from a slightly different point of view.

How did the Reflection activity improve your understanding of what you have studied in Chapter 1?

Final reflection

Discuss with a friend which, if any, parts of Chapter 1 you need to:

- read through again to make sure you really understand
- seek more guidance on, even after going over it again.

SUMMARY

The basic unit of life is the cell. The simplest cells are prokaryotic cells, which are thought to have evolved before, and given rise to, the much more complex and much larger eukaryotic cells.
Cells can be seen clearly only with the aid of microscopes. The light microscope uses light as a source of radiation, whereas the electron microscope uses electrons. The electron microscope has greater resolution (allows more detail to be seen) than the light microscope because electrons have a shorter wavelength than light.
With a light microscope, cells may be measured using an eyepiece graticule and a stage micrometer. Using the formula $A = \frac{I}{M}$ the actual size of an object (A) or its magnification (M) can be found if its observed (image) size (I) is measured and A or M, as appropriate, is known.
All cells are surrounded by a partially permeable cell surface membrane that controls exchange between the cell and its environment. All cells contain genetic material in the form of DNA, and ribosomes for protein synthesis.
All eukaryotic cells possess a nucleus containing DNA. The DNA is linear (not circular) and bound to proteins and RNA to form chromatin.
The cytoplasm of eukaryotic cells contains many organelles, some of which are surrounded by one or two membranes. Organelles of eukaryotic cells include endoplasmic reticulum (ER), 80S ribosomes, Golgi apparatus, lysosomes and mitochondria. Animal cells also contain a centrosome and centrioles and may contain cilia. Plant cells have a cell wall containing cellulose. They may contain chloroplasts and often have a large central vacuole.
Prokaryotic cells lack a true nucleus and have smaller (70S) ribosomes than eukaryotic cells. They also lack membrane-bound organelles. Their DNA is circular and lies free in the cytoplasm.
Viruses do not have a cellular structure. They are extremely small and simple. They consist of a molecule of DNA or RNA, a protein coat and sometimes an outer envelope.

EXAM-STYLE QUESTIONS

1 Which **one** of the following cell structures can be seen with a light microscope?

A mitochondrion
B ribosome
C rough ER
D smooth ER

[1]

2 What property of electrons allows high resolution to be achieved by electron microscopes?

a Electrons are negatively charged.
b Electrons can be focused using electromagnets.
c Electrons have a very short wavelength.
d Electrons travel at the speed of light.

[1]

3 Which **one** of the following structures is found in animal cells but not in plant cells?

A cell surface membrane
B centriole
C chloroplast
D Golgi apparatus

[1]

CONTINUED

4 List **ten** structures you could find in an electron micrograph of an animal cell which would be absent from the cell of a bacterium. **[10]**

5 Distinguish between the following pairs of terms:

- **a** magnification and resolution **[3]**
- **b** light microscope and electron microscope **[2]**
- **c** nucleus and nucleolus **[4]**
- **d** chromatin and chromosome **[3]**
- **e** membrane and envelope **[3]**
- **f** smooth ER and rough ER **[4]**
- **g** prokaryote and eukaryote **[4]**
- **h** cell wall and cell surface membrane **[4]**
- **i** capsid and cell wall **[4]**
- **j** capsid and capsomere **[3]**

[Total: 34]

6 List:

- **a** **three** organelles each lacking a boundary membrane **[3]**
- **b** **three** organelles each surrounded by a single membrane **[3]**
- **c** **three** organelles each surrounded by two membranes (an envelope). **[3]**

[Total: 9]

7 **Identify** each cell structure or organelle from its description below.

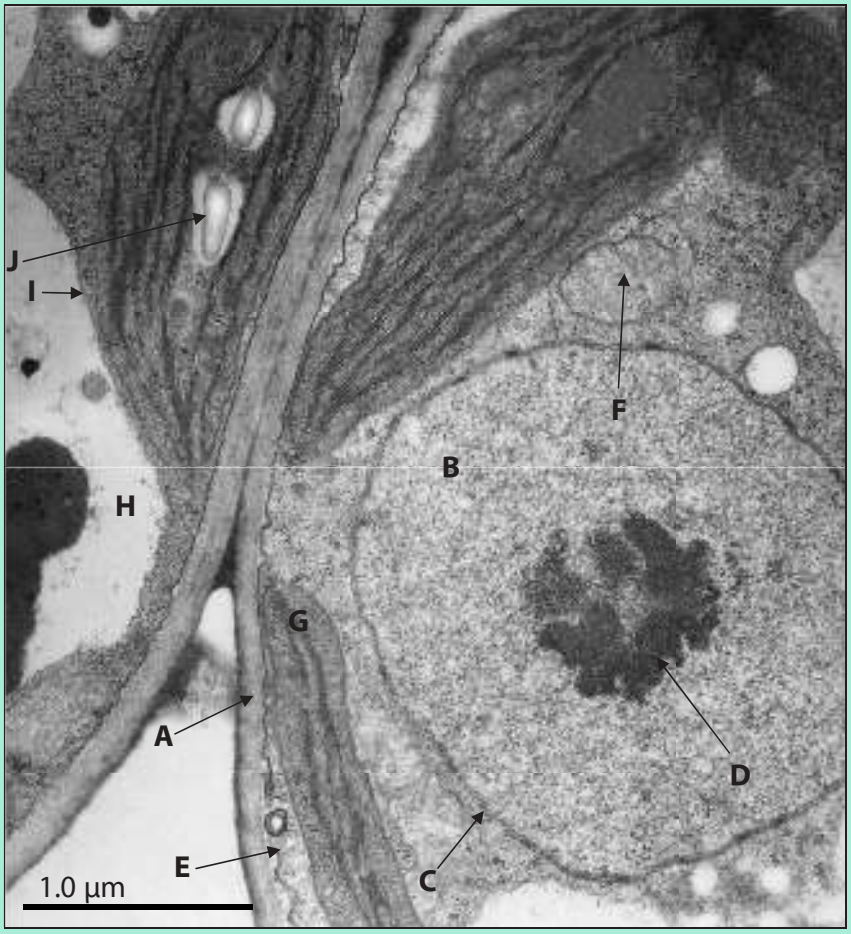
- **a** manufactures lysosomes
- **b** manufactures ribosomes
- **c** site of protein synthesis
- **d** can bud off vesicles which form the Golgi apparatus
- **e** can transport newly synthesised protein round the cell
- **f** manufactures ATP in animal and plant cells
- **g** controls the activity of the cell because it contains the DNA
- **h** carries out photosynthesis
- **i** can act as a starting point for the growth of spindle microtubules during cell division
- **j** contains chromatin
- **k** partially permeable barrier only about 7 nm thick
- **l** organelle about 25 nm in diameter
- **m** organelle with a '9 + 2' arrangement of microtubules **[13]**

COMMAND WORD

Identify: name / select / recognise.

CONTINUED

8 The transmission electron micrograph shows parts of two palisade cells from a leaf.



CONTINUED

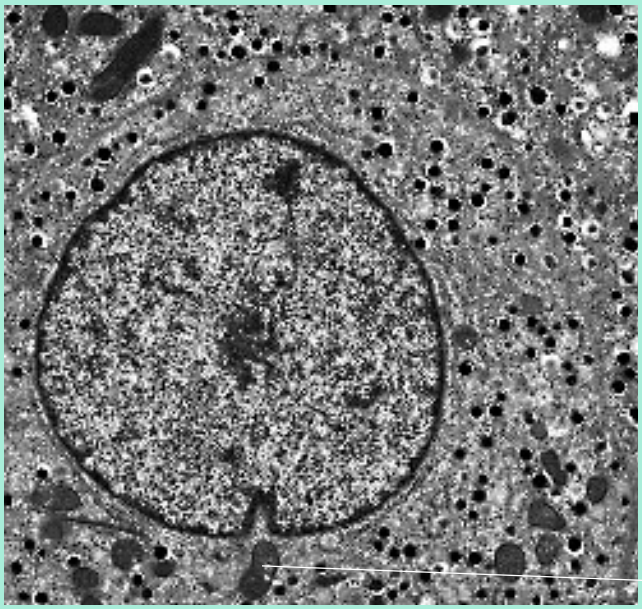
Copy the table. Identify the labelled structures **A–J** and write a brief statement about their functions.

Label	Name of structure	Function	
A			[3]
B			[3]
C			[2]
D			[2]
E			[3]
F			[3]
G			[3]
H			[2]
I			[2]
J			[2]

[Total: 25]

CONTINUED

9 The electron micrograph shows part of a secretory cell from the pancreas. You are not expected to have seen a micrograph of this type of cell before. The cell contains many secretory vesicles. These are Golgi vesicles. They appear as small, roughly circular structures with black circular contents. The magnification is ×8000.



a Copy the table. **Calculate** the actual sizes of the structures listed in the table. Use a ruler with mm divisions to help you. Show your measurements and calculations. When you have your answers, complete the table with the required information. **Give** your answers in micrometres.

Structure	Observed diameter (measured with ruler)	Actual size
maximum diameter of a Golgi vesicle		
maximum diameter of nucleus		
maximum length of the labelled mitochondrion		

[9]

b Make a fully labelled drawing of representative parts of the cell. You do not have to draw everything, but enough to show the structures of the main organelles. Use a full page of plain paper and a sharp pencil. Use Figures 1.18 and 1.19 in this book and the simplified diagram in **d** below to help you identify the structures. **[14]**

c The mitochondria in pancreatic cells are mostly sausage-shaped in three dimensions. **Suggest** why some of the mitochondria in the electron micrograph here appear roughly circular. **[1]**

COMMAND WORDS

Calculate: work out from given facts, figures or information.

Give: produce an answer from a given source or recall/memory.

Suggest: apply knowledge and understanding to situations where there is a range of valid responses in order to make proposals / put forward considerations.

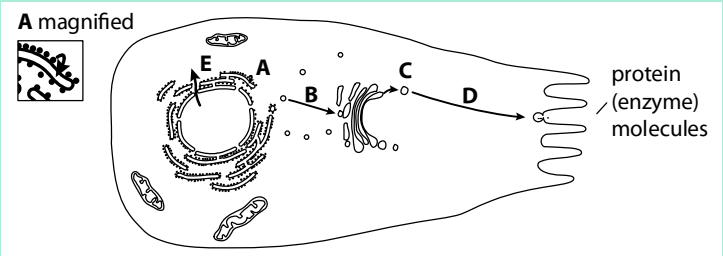
IMPORTANT

Use modelling clay to make a sausage shape to represent a mitochondrion (or use a real sausage). Try cutting the sausage with a knife at different angles. This represents the process of sectioning material for examination using a microscope. The cut surfaces will reveal the variation you can expect to see in sections.

CONTINUED

d The figure is a diagram based on an electron micrograph of a secretory cell from the pancreas. This type of cell is specialised for secreting (exporting) proteins. Some of the proteins are digestive enzymes of the pancreatic juice. The cell is very active, requiring a lot of energy. The arrows **A**, **B**, **C** and **D** show the route taken by the protein molecules.

Note that arrow **A** is shown magnified in a separate diagram.



i Describe briefly what is happening at each of the stages **A**, **B**, **C** and **D**. **[8]**

ii Arrow **E** shows the path of a molecule or structure leaving the nucleus through the nuclear envelope. Name **one** molecule or structure which leaves the nucleus by route **E**. **[1]**

iii The molecule or structure you named in **ii** passes through the nuclear envelope. Name the structure in the nuclear envelope through which the molecule or structure passes. **[1]**

iv Name the molecule which leaves the mitochondrion in order to provide energy for the cell. **[1]**

[Total: 35]

10 One technique used to investigate the activity of cell organelles is called differential centrifugation. In this technique, a tissue is homogenised (ground in a blender), placed in tubes and spun in a centrifuge. This makes organelles sediment (settle) to the bottom of the tubes. The larger the organelles, the faster they sediment. By repeating the process at faster and faster speeds, the organelles can be separated from each other according to size. Some liver tissue was treated in this way to separate ribosomes, nuclei and mitochondria. The centrifuge was spun at 1000 *g*, 10 000 *g* or 100 000 *g* (*g* is gravitational force).

a State in which of the three sediments (1000 *g*, 10 000 *g* or 100 000 *g*) you would expect to find the following:

i ribosomes

ii nuclei

iii mitochondria **[1]**

b Liver tissue contains many lysosomes. Suggest why this makes it difficult to study mitochondria using the differential centrifugation technique. **[4]**

[Total: 5]

COMMAND WORDS

Describe: state the points of a topic / give characteristics and main features.

State: express in clear terms.

SELF-EVALUATION CHECKLIST

After studying this chapter, complete a table like this:

I can	See section...	Needs more work	Almost there	Ready to move on
explain that cells are the basic units of life	1.1			
use the units of measurement relevant to microscopy	1.2			
recognise the common structures found in cells as seen with a light microscope and outline their structures and functions	1.3			
compare the key structural features of animal and plant cells	1.3			
use a light microscope and make temporary preparations to observe cells	1.3			
recognise, draw and measure cell structures from temporary preparations and micrographs	1.3, 1.4			
calculate magnifications of images and actual sizes of specimens using drawings or micrographs	1.4			
explain the use of the electron microscope to study cells with reference to the increased resolution of electron microscopes	1.5			
recognise the common structures found in cells as seen with an electron microscope and outline their structures and functions	1.6			
outline briefly the role of ATP in cells	1.6			
describe the structure of bacteria and compare the structure of prokaryotic cells with eukaryotic cells	1.7, 1.8			
describe the structure of viruses	1.9			

Chapter 2
Biological molecules

LEARNING INTENTIONS

In this chapter you will learn how to:

- describe how large biological molecules are made from smaller molecules
- describe the structure of carbohydrates, lipids and proteins and how their structure relates to their functions
- describe and carry out biochemical tests to identify carbohydrates, lipids and proteins
- explain some key properties of water that make life possible.

BEFORE YOU START

It is useful to remind yourself of how atoms join together to make molecules. The best way to do this is to draw or make models of some simple molecules. You want to show how the carbon, hydrogen, oxygen and nitrogen atoms are joined together with covalent bonds. Carbon has four bonds, nitrogen three, oxygen two and hydrogen one. The bonds should be arranged with the correct orientation (see Figures 2.11, 2.16, 2.23 and 2.27 to help you).

If you can, use model kits. Otherwise, coloured balls of modelling clay (or coloured jelly beans) can be used to represent atoms. Use black for carbon, white for hydrogen, red for oxygen and blue for nitrogen. Join the balls or jelly beans with short sticks such as toothpicks, matchsticks or straws. The sticks represent covalent bonds.

Try making models of or drawing these molecules:

- methane, CH_4
- water, H_2O
- ethanol, C_2H_5OH
- a hydrocarbon, e.g. C_3H_8
- ammonia, NH_3
- ethanoic acid, CH_3COOH.

THE PROTEIN-FOLDING PROBLEM – FROM DEEP BLUE TO ALPHAZERO AND BEYOND

In 1962, two Cambridge scientists, John Kendrew and Max Perutz, received the Nobel Prize for Chemistry for their work on the three-dimensional structure of the proteins myoglobin and haemoglobin. The work was a vital step in understanding how proteins function. Thirty-five years later, in 1997, a world chess champion, Garry Kasparov, was beaten at chess for the first time by the computer Deep Blue. So what is the connection between these two events?

The answer lies in the applications of artificial intelligence (AI). The IBM computer Deep Blue was an important milestone on the road to developing AI. One of the most exciting recent computers to be developed is AlphaZero, the creation of another British scientist, Demis Hassabis. AlphaZero has taught itself to be the best chess player ever. It took only four hours starting from scratch, using the technique known as 'reinforcement learning' – learning by trial and error by playing millions of games against itself.

How can a computer like this be of use to humans? There are many problems in the world, such as climate change, which are too complex for the

For example, Demis Hassabis has suggested that, in the future, AlphaZero and computers like it may be able to design more effective drugs and medicines. One of the key problems in biology is the so-called 'protein-folding problem'. This is the problem of trying to discover the rules of how proteins fold into the precise three-dimensional shapes essential for their functions. Ideally, knowing the primary structure of a protein and its chemical environment (e.g. pH and temperature) would enable scientists to predict how the protein will fold up. The work has vital applications. For example,

problem.

CONTINUED

many diseases and disorders including Alzheimer's, Parkinson's and cystic fibrosis are caused by faulty protein folding. In December 2018, the computer AlphaFold won an international contest to predict protein structure more accurately than previous attempts.

Around 60 years after the pioneering work of Kendrew and Perutz, scientists are getting closer to the goal of predicting how proteins will fold, but it seems only AI can provide all the answers (Figure 2.1).

Questions for discussion

Can you think of any potential problems with AI? Do you think the benefits outweigh these problems, or not?

2.1 Biochemistry

Biochemistry looks at the chemical reactions of biological molecules. The sum total of all the biochemical reactions in the body is known as metabolism. You may think biochemistry is a complicated subject, but it has an underlying simplicity. For example, only 20 common amino acids are used to make proteins, whereas theoretically there could be millions. Having a limited variety of molecules makes it easier to control metabolism.

Another feature of biochemistry is the close link between the structures of molecules and their functions. This will become clear in this chapter and in Chapter 3.

2.2 The building blocks of life

The four most common elements in living organisms are, in order of abundance, hydrogen, carbon, oxygen and nitrogen. They account for more than 99% of the atoms found in all living things. Carbon is particularly important because carbon atoms can join together to form long chains or ring structures. They can be thought of as the basic skeletons of organic molecules (molecules that contain carbon). Other atoms, with different functions, are attached to the carbon skeletons.

It is believed that, before life evolved, there was a period of chemical evolution in which simple carbon-based biological molecules evolved from even simpler molecules. The simple biological molecules are relatively limited in variety. They act as the building blocks for larger, complex biological molecules (Figure 2.2).

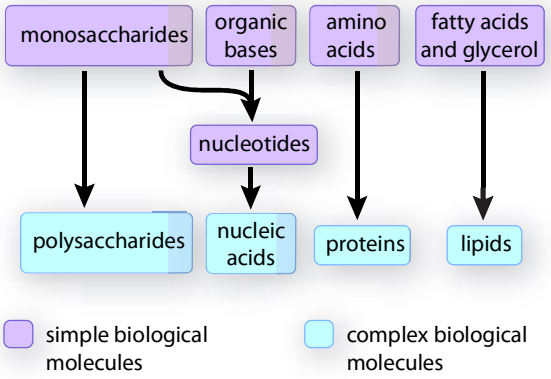


Figure 2.2: The building blocks of life are simple biological molecules which join together to form larger more complex molecules.

2.3 Monomers, polymers and macromolecules

A **macromolecule** is a giant molecule. There are three types of macromolecule in living organisms:

- polysaccharides
- proteins (polypeptides)
- nucleic acids (polynucleotides).

KEY WORD

macromolecule: a large molecule such as a polysaccharide, protein or nucleic acid

The words polysaccharides, polypeptides and polynucleotides all contain the term *poly*. 'Poly' means many. Macromolecules are described as **polymers** because they are made up of many repeating subunits that are similar or identical to each other. The subunits are called **monomers** ('mono' means one). Monomers are joined together by covalent bonds. These are bonds in which the atoms are joined by sharing electrons. Covalent bonds are relatively strong bonds. Examples you will learn about in this chapter are the glycosidic bond, the ester bond and the peptide bond.

Making biological polymers from monomers is relatively simple because the same reaction is repeated many times. The reaction involves joining together two monomers by the *removal* of a water molecule. This type of reaction is called a **condensation reaction**. The opposite reaction (*adding* water) can be used to break down the polymer again. Adding water to split a molecule is called **hydrolysis**. You will meet many examples of condensation and hydrolysis in this chapter.

The monomers from which polysaccharides, proteins and nucleic acids are made are monosaccharides, amino acids and nucleotides respectively (Figure 2.2.) Figure 2.2 also shows the role of organic bases (not monomers) in nucleotides and the role of fatty acids and glycerol in the formation of lipids (not polymers).

Cellulose and rubber are examples of naturally occurring polymers. There are many examples of industrially produced polymers, such as polyester, polythene, PVC (polyvinyl chloride) and nylon. All these are made up of carbon-based monomers and contain thousands of carbon atoms joined end to end.

Let's now look at some of the small biological molecules (monosaccharides, fatty acids and amino acids) and the larger molecules (carbohydrates, lipids and proteins) made from them. Organic bases, nucleotides and nucleic acids are discussed in Chapter 6.

KEY WORDS

polymer: a giant molecule made from many similar repeating subunits joined together in a chain; the subunits are much smaller and simpler molecules known as monomers; examples of biological polymers are polysaccharides, proteins and nucleic acids

monomer: a relatively simple molecule which is used as a basic building block for the synthesis of a polymer; many monomers are joined together by covalent bonds to make the polymer, usually by condensation reactions; common examples of monomers are monosaccharides, amino acids and nucleotides

condensation reaction: a chemical reaction involving the joining together of two molecules by removal of a water molecule

hydrolysis: a chemical reaction in which a chemical bond is broken by the addition of a water molecule; commonly used to break down

2.4 Carbohydrates

All carbohydrates contain the elements carbon, hydrogen and oxygen. The 'hydrate' part of the name refers to water; the hydrogen and oxygen atoms are present in the ratio of 2 : 1 as in water. The general formula for a carbohydrate can be written as $C_x(H_2O)_y$.

Carbohydrates are divided into three main groups: monosaccharides, disaccharides and polysaccharides. The word 'saccharide' means a sugar or sweet substance.

Monosaccharides

Monosaccharides are sugars. Sugars dissolve easily in water to form sweet-tasting solutions. Monosaccharides consist of a single sugar molecule ('mono' means one, 'saccharide' means sugar). They have the general formula $(CH_2O)_n$. The main types of monosaccharides (when classified according to the number of carbon atoms in each molecule) are trioses (3C), pentoses (5C) and hexoses (6C). The names of all sugars end with -ose. Common hexoses are glucose, fructose and galactose. Two common pentoses are ribose and deoxyribose.

Molecular and structural formulae

The molecular formula for a hexose can be written as $C_6H_{12}O_6$. It means there are 6 carbon atoms, 12 ı atoms in the molecule. It nents of the atoms using tural formula. Figure 2.3 of glucose, the most lucose is a hexose.

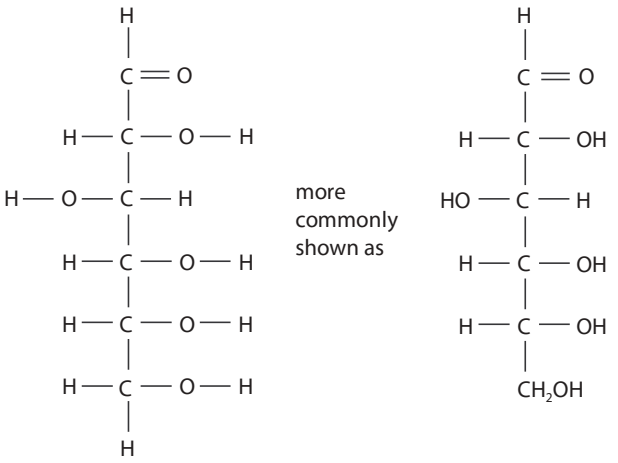


Figure 2.3: Structural formula of glucose. –OH is known as a hydroxyl group. There are five in glucose.

Ring structures

One important aspect of the structure of pentoses and hexoses is that the chain of carbon atoms is long enough to close up on itself to form a more stable ring structure. When glucose forms such a ring, carbon atom number 1 joins to the oxygen on carbon atom number 5 (Figure 2.4). The ring therefore contains oxygen, and carbon atom number 6 is not part of the ring.

You will see from Figure 2.4 that the hydroxyl group, –OH, on carbon atom 1 may be above or below the plane of the ring. The form of glucose where it is below the ring is known as α-glucose (alpha-glucose) and the form where it is above the ring is β-glucose (beta-glucose). Two forms of the same chemical are known as isomers, and the extra variety provided by the existence of α- and β-isomers has important biological consequences, as you will see in the structures of starch, glycogen and cellulose.

Question

1 The formula for a hexose is $C_6H_{12}O_6$ or $(CH_2O)_6$. What would be the formula of:
   a a triose?
   b a pentose?

Functions of monosaccharides in living organisms

Monosaccharides have two major functions. First, they are commonly used as a source of energy in respiration. This is due to the large number of carbon–hydrogen bonds. These bonds can be broken to release

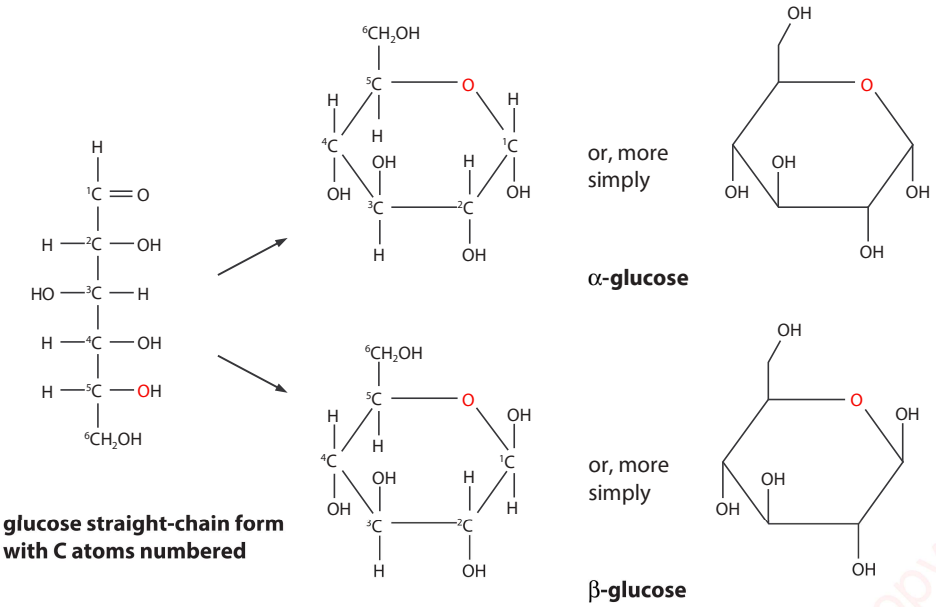


Figure 2.4: Structural formulae for the straight-chain and ring forms of glucose. Chemists often leave out the C and H atoms from the structural formula for simplicity.

a lot of energy, which is transferred to help make ATP (adenosine triphosphate) from ADP (adenosine diphosphate) plus phosphate during the process of respiration. The most important monosaccharide in energy metabolism is glucose.

Second, monosaccharides are important as building blocks for larger molecules. For example, glucose is used to make the polysaccharides starch, glycogen and cellulose. Ribose (a pentose) is one of the molecules used to make RNA (ribonucleic acid) and ATP. Deoxyribose (also a pentose) is one of the molecules used to make DNA (Chapter 6).

Disaccharides and the glycosidic bond

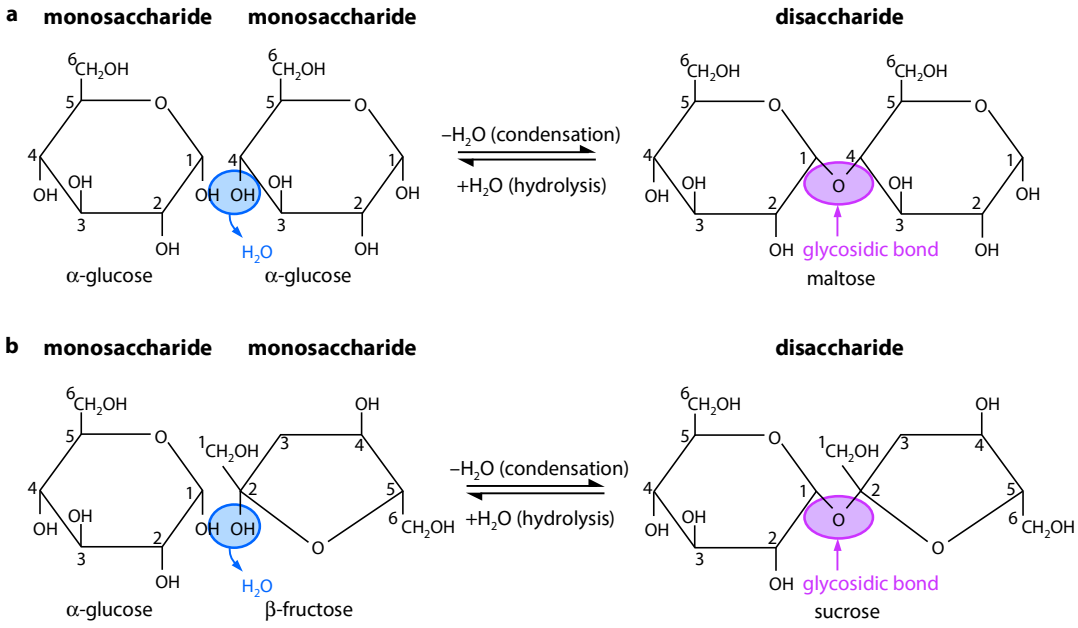
Disaccharides, like monosaccharides, are sugars. They are formed by two monosaccharides joining together ('di' means two). The three most common disaccharides are maltose (glucose + glucose), sucrose (glucose + fructose) and lactose (glucose + galactose). Sucrose is the transport sugar in plants and the sugar commonly bought in shops. Lactose is the sugar found in milk and is therefore an important constituent of the diet of young mammals.

KEY WORD

disaccharide: a sugar molecule consisting of two monosaccharides joined together by a glycosidic bond

The process of joining two monosaccharides is an example of a condensation reaction (Figure 2.5; see also Section 2.3, Monomers, polymers and macromolecules). The reverse process (splitting a disaccharide into two monomers) is also shown in Figure 2.5 and is an example of a hydrolysis reaction. Notice that fructose has a different ring structure from glucose.

For each condensation reaction, two hydroxyl (–OH) groups line up alongside each other. One combines with a hydrogen atom from the other to form a water molecule. This allows an oxygen 'bridge' to form between the two molecules, holding them together



is formed from two
is example the glycosidic
made from an α-glucose

and forming a disaccharide. The bridge is called a **glycosidic bond**.

In theory, any two –OH groups can line up and, since monosaccharides have many –OH groups, there are a large number of possible disaccharides. The shape of the enzyme controlling the reaction determines which –OH groups come alongside each other. Only a few of the possible disaccharides are common in nature.

The addition of water in hydrolysis takes place during the digestion of disaccharides and polysaccharides, when they are broken down to monosaccharides.

KEY WORD

glycosidic bond: a C–O–C link between two sugar molecules, formed by a condensation reaction; it is a covalent bond

PRACTICAL ACTIVITY 2.1

Testing for the presence of sugars

1 Reducing sugars

Reducing sugars are so called because they can carry out a type of chemical reaction known as reduction. In the process they are oxidised. The reducing sugars include all monosaccharides and some disaccharides. The only common non-reducing sugar is sucrose.

The ability of some sugars to carry out reduction is the basis of **Benedict's test** for the presence of sugar. The test uses Benedict's reagent which is copper(II) sulfate in an alkaline solution. It has a distinctive blue colour. Reducing sugars reduce the soluble blue copper sulfate to insoluble brick-red copper oxide, containing copper(I). The copper oxide is seen as a brick-red precipitate.

reducing sugar + Cu^{2+} → oxidised sugar + Cu^{+}

blue red-brown

Procedure

Add Benedict's reagent to the solution you are testing and heat it in a water bath. If a reducing sugar is present, the solution will gradually turn through green, yellow and orange to red-brown as the insoluble copper(I) oxide forms a precipitate.

KEY WORD

Benedict's test: a test for the presence of reducing sugars; the unknown substance is heated with Benedict's reagent, and a change from a clear blue solution to the production of a yellow, red or brown precipitate indicates the presence of reducing sugars such as glucose

As long as you use excess Benedict's reagent (more than enough to react with all of the sugar present), the intensity of the red colour is related to the concentration of the reducing sugar. The test can therefore be used as a semi-quantitative test. You can estimate the concentration of a reducing sugar solution using colour standards made by comparing the colour against the colours obtained in tests done with reducing sugar solutions of known concentration. You could also measure the time taken for the first colour change.

Alternatively, you can use a colorimeter to measure small differences in colour more precisely.

2 Non-reducing sugars

Some disaccharides, such as sucrose, are not reducing sugars, so you would get a negative result from Benedict's test. In such a case, you should then carry out the test for a non-reducing sugar.

In the non-reducing sugars test, the disaccharide is first broken down into its two monosaccharide constituents. The chemical reaction is hydrolysis and can be brought about by adding hydrochloric acid. The constituent monosaccharides will be reducing sugars and their presence can be tested for using Benedict's test after the acid has been neutralised.

Procedure

Carry out Benedict's test on the solution. If you get a negative result, start again with a fresh sample of the solution. Heat the solution with hydrochloric acid. If a non-reducing sugar is present, it will break down to monosaccharides. Benedict's reagent needs alkaline conditions to work, so you need to neutralise the test solution now by adding an alkali such as sodium

CONTINUED

hydroxide. Add Benedict's reagent and heat as before and look for the colour change. If the solution now goes red, a non-reducing sugar is present. If both a reducing sugar and a non-reducing sugar are present, the precipitate will be heavier than the one obtained in the Benedict's test. If there is still no colour change, then there is no sugar of any kind present.

(See Practical Investigation 2.1 in the Practical Workbook for additional information.)

Question

2 a Why do you need to use excess Benedict's reagent to find the concentration of a sugar solution?

b Outline how you could use the Benedict's test to estimate the concentration of a solution of a reducing sugar.

Polysaccharides

Polysaccharides are polymers made by joining many monosaccharide molecules by condensation. Each successive monosaccharide is added by means of a glycosidic bond, as in disaccharides. The final molecule may be several thousand monosaccharide units long, forming a macromolecule. The most important polysaccharides are starch, glycogen and cellulose, all of which are polymers of glucose. Polysaccharides are not sugars.

Starch and glycogen

Since glucose is the main source of energy for cells, it is important for living organisms to store glucose in an appropriate form. If glucose itself accumulated in cells, it would dissolve and make the contents of the cell too concentrated. This would seriously affect the osmotic properties of the cell (Chapter 4, Section 4.5, Movement of substances across membranes). Glucose is also a reactive molecule and would interfere with normal cell chemistry. These problems are avoided when glucose is converted by condensation reactions to a storage polysaccharide. The storage polysaccharide is a convenient, compact, inert (unreactive) and insoluble molecule. The storage polysaccharide in plants is starch; in animals, it is **glycogen**. When needed, glucose is quickly made available again by

they are linked between carbon atoms 1 and 4 of successive glucose units.) The chains are curved (Figure 2.6) and coil up into helical structures like springs, so the final molecule is compact.

Amylopectin is also made of many 1,4 linked α-glucose molecules, but the chains are shorter than in amylose and also contain 1,6 linkages. These start branches out to the sides of the chain (Figure 2.7).

Mixtures of amylose and amylopectin molecules build up into relatively large starch grains. Starch grains are commonly found in chloroplasts and in storage organs, such as potato tubers and the seeds of cereals and legumes (Figure 2.8). Starch grains are easily seen with a

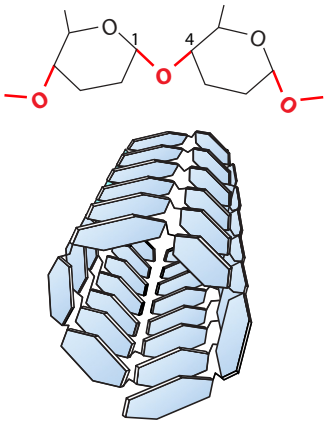


Figure 2.6: Arrangement of α-glucose units in amylose. The 1,4 linkages cause the chain to turn and coil. The glycosidic bonds are shown in red and the hydroxyl groups are omitted.

KEY WORDS

polysaccharide: a polymer whose subunits are ogether by glycosidic

de made of many together, that acts as a muscle cells

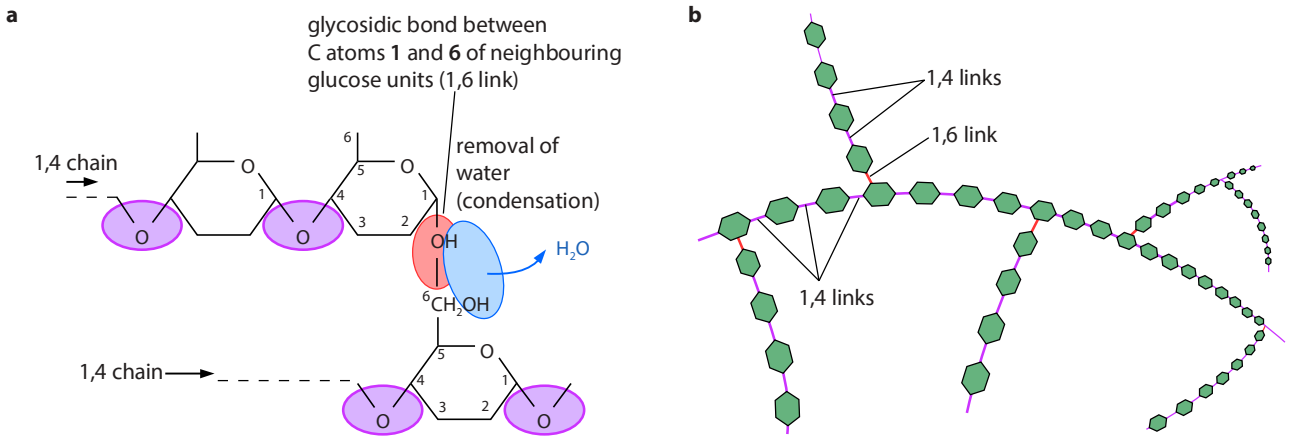


Figure 2.7: Branching structure of amylopectin and glycogen. **a** Formation of a 1,6 link, making a branchpoint; **b** overall structure of an amylopectin or glycogen molecule. Amylopectin and glycogen differ only in the amount of branching of their glucose chains; glycogen is more branched than amylopectin.

light microscope, especially if stained. Rubbing a freshly cut potato tuber on a glass slide and staining with iodine–potassium iodide solution (Practical Activity 2.2) is a quick method of preparing a specimen for viewing.

Figure 2.8: False-colour scanning electron micrograph of a slice through a raw potato showing cells containing starch grains or starch-containing organelles (coloured red) (×260).

Starch is never found in animal cells. Glycogen is the storage carbohydrate in animals. It has molecules very like those of amylopectin because it is made of chains of 1,4 linked α-glucose with 1,6 linkages making branch points (Figure 2.7b). Glycogen molecules clump together to form granules, which are visible in liver cells (see Figure 1.18) and muscle cells, where they form an energy reserve.

Questions

3 What type of chemical reaction happens when glucose is formed from starch or glycogen?

4 List **five** ways in which the molecular structures of glycogen and amylopectin are similar.

PRACTICAL ACTIVITY 2.2

Testing for the presence of starch

Starch molecules tend to curl up into long spirals. The hole that runs down the middle of this spiral is just the right size for iodine molecules to fit into. To test for starch, you use 'iodine solution'. (Iodine doesn't dissolve in water; iodine solution is actually iodine in potassium iodide solution.) The starch–iodine complex that forms has a strong blue-black colour.

Procedure

Iodine solution is orange-brown. Add a drop of iodine solution to the solid or liquid substance to be tested. A blue-black colour is quickly produced if starch is present.

(See Practical Investigation 2.1 in the Practical Workbook for additional information.)

Cellulose

Cellulose is the most abundant organic molecule on the planet. This is due to its presence in plant cell walls and its slow rate of breakdown in nature. It has a structural role because it is a mechanically strong molecule, unlike starch and glycogen. The only difference is that cellulose is a polymer of β-glucose, and starch and glycogen are polymers of α-glucose.

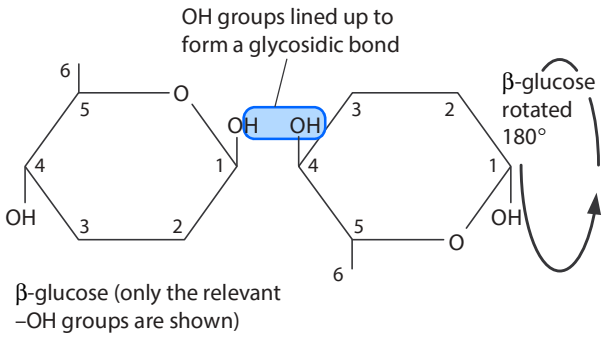
KEY WORD

cellulose: a polysaccharide made from beta-glucose subunits; used as a strengthening material in plant cell walls

Remember that the –OH group on carbon atom 1 projects above the ring in the β-isomer of glucose (Figure 2.4). In order to form a glycosidic bond with carbon atom 4, where the –OH group is below the ring, one glucose molecule must be upside down (rotated 180°) relative to the other. Thus successive glucose units are linked at 180° to each other (Figure 2.9).

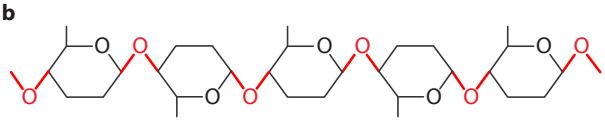
This arrangement of β-glucose molecules results in a strong molecule because the hydrogen atoms of –OH groups are weakly attracted to oxygen atoms in the same cellulose molecule (the oxygen of the glucose ring) and also to oxygen atoms of –OH groups in neighbouring molecules. These hydrogen bonds are individually weak, but there are so many of them (due to the large number of –OH groups) that collectively they provide enormous strength. Between 60 and 70 cellulose molecules become tightly cross-linked by hydrogen bonding to form bundles called microfibrils. Microfibrils are in turn held together by hydrogen bonding in bundles called fibres.

a



β-glucose (only the relevant –OH groups are shown)

b



A cell wall typically has several layers of fibres, running in different directions to increase strength (Figure 2.10). Cellulose makes up about 20–40% of the average cell wall; other molecules help to cross-link the cellulose fibres, and some form a glue-like matrix around the fibres, which further increases strength.

Cellulose fibres have a very high tensile strength, almost equal to that of steel. This means that, if pulled at both ends, they are very difficult to stretch or break. The high tensile strength of the cellulose fibres makes it possible for a cell to withstand the large pressures that develop within it as a result of osmosis (Chapter 4, Section 4.5, Movement of substances across membranes). Without the wall, the cell would burst when in a dilute solution. These pressures help provide support for the plant by making tissues rigid, and are responsible for cell expansion during growth. The arrangement of fibres around the cell helps to determine the shape of the cell as it grows.

Despite their strength, cellulose fibres are freely permeable, allowing water and solutes to reach or leave the cell surface membrane.

Question

5 Make a table to show **three** ways in which the molecular structures of amylose and cellulose differ.

Dipoles and hydrogen bonds

When atoms in molecules are held together by covalent bonds, they share electrons with each other. Each shared pair of electrons forms one covalent bond. For example, in a water molecule, two hydrogen atoms each share a pair of electrons with an oxygen atom, forming a

$_2O$.

n

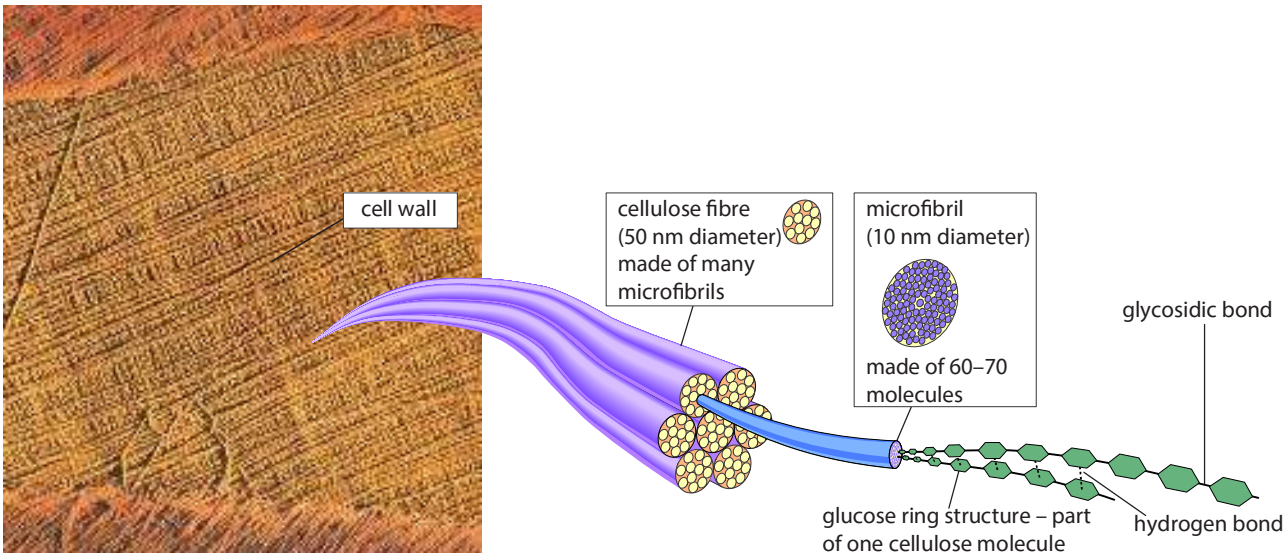
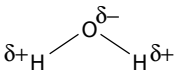


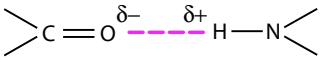
Figure 2.10: Structure of cellulose.

However, the electrons are not shared absolutely equally. In water, the oxygen atom gets slightly more than its fair share, and so has a small negative charge, written δ− (delta minus). The hydrogen atoms get slightly less than their fair share, and so have a small positive charge, written δ+ (delta plus).

This unequal distribution of charge is called a dipole:



In water, the negatively charged oxygen of one molecule is attracted to a positively charged hydrogen of another, and this attraction is called a **hydrogen bond**. The hydrogen bond is traditionally shown as a dotted or dashed line in diagrams:



It is much weaker than a covalent bond, but still has a very significant effect. You will find out how hydrogen bonds affect the properties of water in Section 2.7, Water.

Dipoles occur in many different molecules, particularly where there is an –OH, –CO or –NH group. Hydrogen bonds can form between these groups, because the negatively charged part of one group is attracted to the positively charged part of another. These bonds are very important in the structure and properties of carbohydrates and proteins.

Molecules that have groups with dipoles, such as sugars, are said to be polar. Polar molecules are attracted to water molecules because the water molecules also have dipoles. Such molecules are hydrophilic (water-loving), and tend to be soluble in water. Molecules which do not have dipoles are said to be non-polar. They are not attracted to water, and so, are hydrophobic (water-hating). Such properties make possible the formation of cell membranes (Chapter 4).

KEY WORD

hydrogen bond: a relatively weak bond formed by the attraction between a group with a small positive charge on a hydrogen atom ($H^{\delta+}$) and another group carrying a small negative charge ($^{\delta-}$), e.g. between two $–O^{\delta-}$ $H^{\delta+}$ groups

2.5 Lipids

Lipids are a very varied group of chemicals. They are all organic molecules which are insoluble in water. Most lipids are formed by fatty acids combining with an alcohol. The most familiar lipids are fats and oils. Fats are solid at room temperature and oils are liquid at room temperature, but chemically they are very similar.

Fatty acids

Fatty acids are a series of acids, some of which are found in lipids. They contain the acidic group –COOH, known as a carboxyl group. The carboxyl group forms the 'head' of the fatty acid molecule. The common fatty acids have long hydrocarbon tails attached to the carboxyl group (Figure 2.11). As the name suggests, the hydrocarbon tail consists of a chain of carbon atoms combined with hydrogen. The chain is often 15 or 17 carbon atoms long.

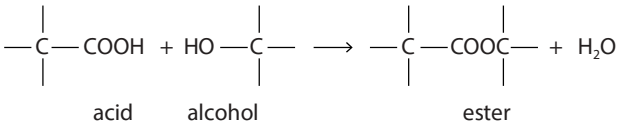
The tails of some fatty acids have double bonds between neighbouring carbon atoms, like this: –C=C–. Such fatty acids are described as unsaturated because they do not contain the maximum possible amount of hydrogen. They form unsaturated lipids. Double bonds make fatty acids and lipids melt more easily – for example, most oils are unsaturated. If there is more than one double bond, the fatty acid or lipid is described as polyunsaturated; if there is only one, it is monounsaturated.

Animal lipids are often saturated (no double bonds) and occur as fats, whereas plant lipids are often unsaturated and occur as oils, such as olive oil and sunflower oil.

Alcohols and esters

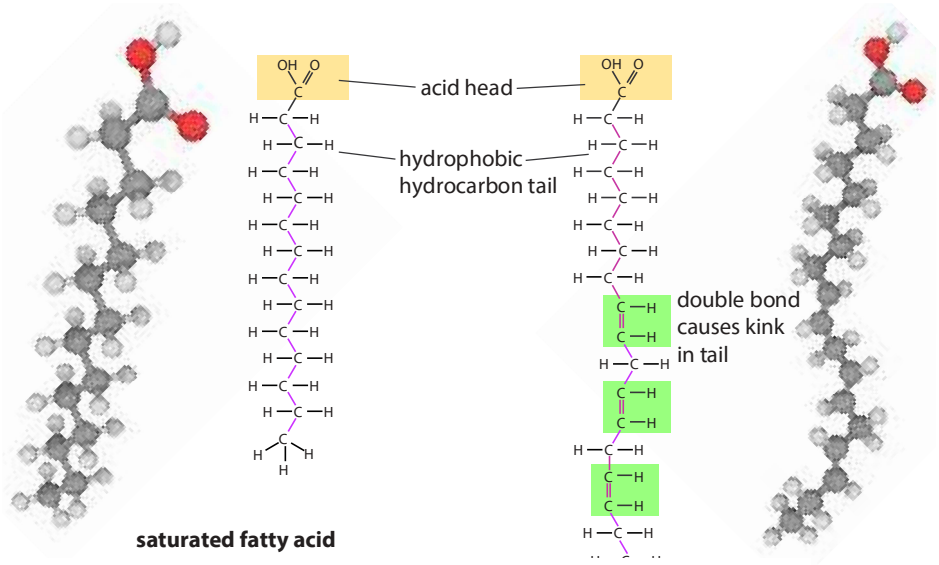
Alcohols are a series of organic molecules which contain a hydroxyl group, –OH, attached to a carbon atom. Glycerol is an alcohol with three hydroxyl groups (Figure 2.12).

The reaction between an acid and an alcohol produces a chemical known as an ester. The chemical link between the acid and the alcohol is called an **ester bond** or an **ester linkage**.



KEY WORD

ester bond / ester linkage: a chemical bond, represented as –COO– , formed when an acid reacts with an alcohol



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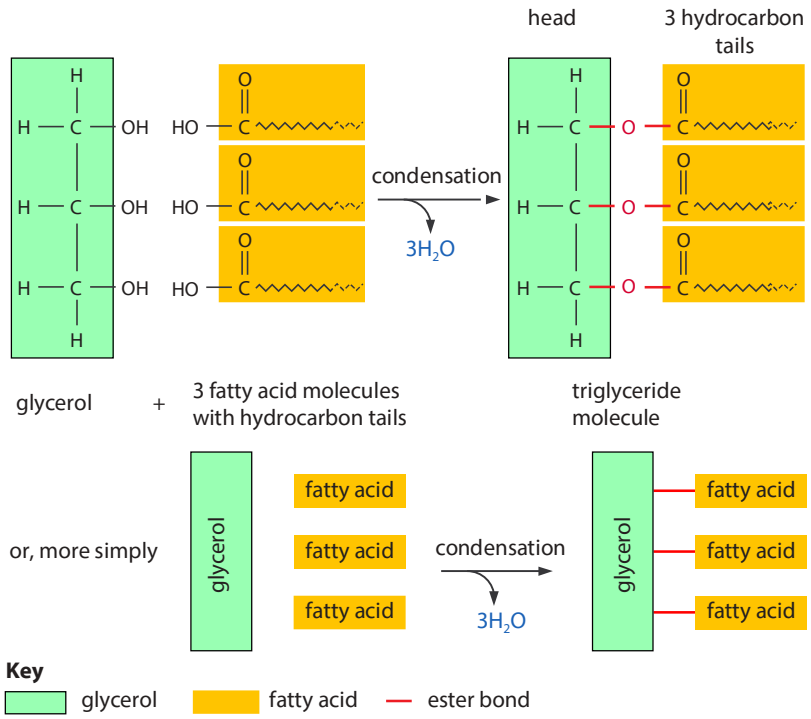

Figure 2.12: Formation of a triglyceride from glycerol and three fatty acid molecules.

The –COOH group on the acid reacts with the –OH group on the alcohol to form the ester bond, –COO– . This is a condensation reaction because water is formed as a product. The resulting ester can be converted back to acid and alcohol by the reverse reaction of adding water, a reaction known as hydrolysis.

Triglycerides

The most common lipids are **triglycerides** (Figure 2.13). These are fats and oils. A glyceride is an ester formed by a fatty acid combining with the alcohol glycerol. As you have seen, glycerol has three hydroxyl groups. Each one is able to undergo a condensation reaction with a fatty acid. When a triglyceride is made, as shown in Figure 2.12, the final molecule contains three fatty acid tails and three ester bonds ('tri' means three). The tails can vary in length, depending on the fatty acids used.

Triglycerides are insoluble in water but are soluble in certain organic solvents such as ethanol. This is because the hydrocarbon tails are non-polar: they have no uneven distribution of electrical charge.

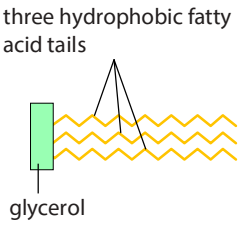

Figure 2.13: Diagrammatic representation of a triglyceride molecule.

Consequently, they are hydrophobic and do not mix freely with water molecules. Figure 2.13 shows a simplified diagram of a triglyceride.

KEY WORD

triglyceride: a type of lipid formed when three fatty acid molecules combine with glycerol, an alcohol with three hydroxyl (–OH) groups

Functions of triglycerides

Triglycerides make excellent energy stores because they are even richer in carbon–hydrogen bonds than carbohydrates. A given mass of triglyceride will therefore yield more energy on oxidation than the same mass of carbohydrate (it has a higher calorific value), an important advantage for a storage product.

Triglycerides are stored in a number of places in the human body, particularly just below the skin and around the kidneys. Below the skin they also act as an insulator against loss of heat. Blubber, a triglyceride found in sea mammals such as whales, has a similar function, as well as providing buoyancy.

An unusual role for triglycerides is as a metabolic source of water. When oxidised in respiration, triglycerides are converted to carbon dioxide and water. The water may be of importance in very dry habitats. For example, the desert kangaroo rat (Figure 2.14) never drinks water and survives on metabolic water from the triglyceride-containing foods it eats.

Figure 2.14: The desert kangaroo rat uses metabolism of food to provide the water it needs.

Phospholipids

Phospholipids are a special type of lipid. Each molecule has the unusual property of having one end

The two remaining hydrocarbon tails are still hydrophobic (Figure 2.15). This allows phospholipids to form a membrane around a cell; two rows of phospholipids are arranged with their hydrophilic heads in the watery solutions on either side of the membrane and their hydrophobic tails forming a layer that is impermeable to hydrophilic substances. The biological significance of this will become apparent when you study membrane structure (Chapter 4).

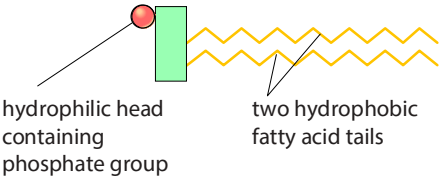

Figure 2.15: Diagrammatic representation of a phospholipid molecule. Compare this with Figure 2.13.

PRACTICAL ACTIVITY 2.3

Testing for the presence of lipids

Lipids are insoluble in water, but soluble in ethanol (alcohol). This fact is made use of in the emulsion test for lipids.

Procedure

The substance that is thought to contain lipids is shaken vigorously with some absolute ethanol (ethanol with little or no water in it). This allows any lipids in the substance to dissolve in the ethanol. The ethanol is then poured into a tube containing water. If lipid is present, a cloudy white suspension is formed.

Further information

If there is no lipid present, the ethanol just mixes into the water. Light can pass straight through this mixture, so it looks completely transparent. But if there is lipid dissolved in the ethanol, it cannot remain dissolved when mixed with the water. The lipid molecules form tiny droplets throughout the liquid. This kind of mixture is called an emulsion.

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1 2.1 in the Practical nformation.)

2.6 Proteins

Proteins are an extremely important class of macromolecule in living organisms. More than 50% of the dry mass of most cells is protein. Proteins have many important functions:

- all enzymes are proteins
- proteins are essential components of cell membranes – their functions in membranes are discussed in Chapter 4
- some hormones are proteins – for example, insulin and glucagon
- the oxygen-carrying pigments haemoglobin and myoglobin are proteins
- antibodies, which attack and destroy invading microorganisms, are proteins
- collagen is a protein that adds strength to many animal tissues – for example, bone and the walls of arteries
- hair, nails and the surface layers of skin contain the protein keratin
- actin and myosin are the proteins responsible for muscle contraction
- proteins may be storage products – for example, casein in milk and ovalbumin in egg white.

Despite their tremendous range of functions, all proteins are made from the same basic monomers. These are amino acids.

Amino acids

Figure 2.16 shows the general structure of all amino acids and the structure of glycine, the simplest amino acid. All amino acids have a central carbon atom which is bonded to an amino group, $–NH_2$, and a carboxylic acid group, –COOH. These two groups give amino acids their name. The third component that is always bonded to the carbon atom is a hydrogen atom. So, the only way in which amino acids differ from each other is in the fourth group of atoms bonded to the central carbon. This is called the R group.

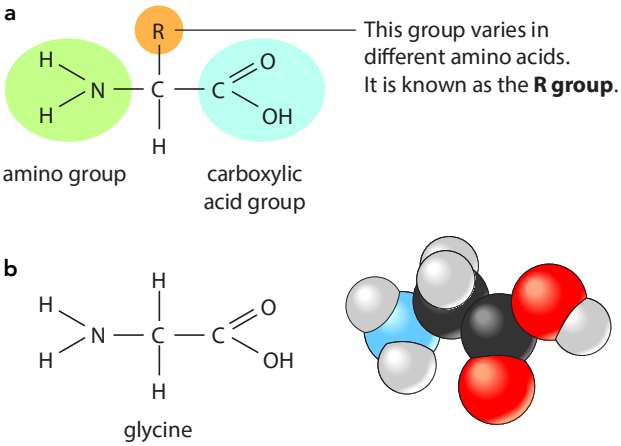


Figure 2.16: a The general structure of an amino acid; **b** structure of the simplest amino acid, glycine, in which the R group is H, hydrogen.

The R groups for the 20 different amino acids which occur in the proteins of living organisms are shown in Appendix 1. You do not need to remember these. Appendix 1 also shows the three-letter abbreviations for the names of the amino acids. Many other amino acids have been synthesised in laboratories.

The peptide bond

Figure 2.17 shows how two amino acids can join together. One loses a hydroxyl (–OH) group from its carboxylic acid group, while the other loses a hydrogen atom from its amino group. This leaves a carbon atom of the first amino acid free to bond with the nitrogen atom of the second. The link is called a **peptide bond**. The oxygen and two hydrogen atoms removed from the amino acids form a water molecule. You have seen condensation reactions like this in the formation of glycosidic bonds (Figure 2.5) and in the synthesis of triglycerides (Figure 2.12).

KEY WORD

peptide bond: the covalent bond joining neighbouring amino acids together in proteins; it is a C–N link between two amino acid molecules, formed by a condensation reaction

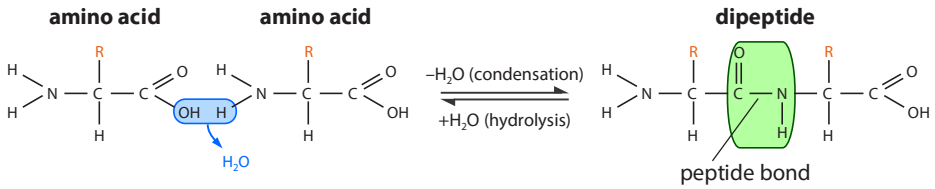


Figure 2.17: Amino acids link together by the loss of a molecule of water to form a peptide bond.

The new molecule is made up of two linked amino acids and is called a dipeptide. A molecule made up of many amino acids linked together by peptide bonds is called a **polypeptide**. A polypeptide is another example of a polymer and a macromolecule, like a polysaccharide. A protein may have just one polypeptide chain or it may have two or more chains.

In living cells, protein synthesis takes place at ribosomes (Chapter 6).

Proteins can be broken down to amino acids by breaking the peptide bonds. This is a hydrolysis reaction, involving the addition of water (Figure 2.17). It happens naturally in the stomach and small intestine during digestion of proteins in food. The amino acids released are absorbed into the blood.

Primary structure

A polypeptide or protein molecule may contain several hundred amino acids linked into a long chain. The particular amino acids contained in the chain, and the sequence in which they are joined, is called the **primary structure** of the protein (Figure 2.18).

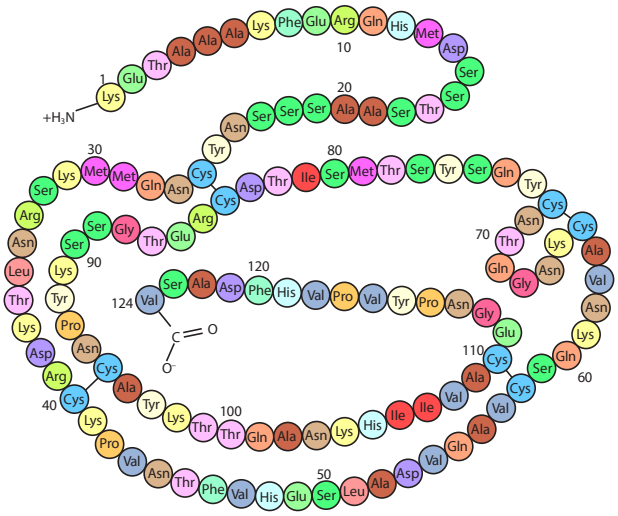


Figure 2.18: The primary structure of ribonuclease. Ribonuclease is an enzyme found in pancreatic juice, which hydrolyses (digests) RNA (Chapter 6). Notice that at one end of the amino acid chain there is an amino group ($-NH_3^+$), while at the other end there is a carboxyl group ($-COO^-$). These are known as the amino and carboxyl ends or the N and C terminals respectively. Note the three-letter abbreviations for the amino acids. These are explained in Appendix 1.

KEY WORDS

polypeptide: a long chain of amino acids formed by condensation reactions between the individual amino acids; proteins are made of one or more polypeptide chains; *see* peptide bond

primary structure: the sequence of amino acids in a polypeptide or protein

There are an enormous number of different possible primary structures. A change in a single amino acid in a chain made up of thousands may completely alter the properties of the polypeptide or protein.

Secondary structure

The amino acids in a polypeptide chain may have an
hey are not next to each
e of amino acids. This is
n can bend back on itself.
of it, often coils into a
condary structure called

an **α-helix** (Figure 2.19a). This secondary structure is due to hydrogen bonding between the oxygen of the C=O group of one amino acid and the hydrogen of the –NH group of the amino acid four places ahead of it. Each amino acid has an –NH and a C=O group, and Figure 2.19a shows that all these groups are involved in hydrogen bonding in the α-helix, holding the structure firmly in shape. Hydrogen bonding is a result of the polar characteristics of the C=O and –NH groups.

Sometimes hydrogen bonding can result in a much looser, straighter shape than the α-helix, which is called a **β-pleated sheet** (Figure 2.19b). Although hydrogen bonds are strong enough to hold the α-helix and β-pleated sheet structures in shape, they are easily broken by high temperatures and pH changes. As you will see, this has important consequences for living organisms.

KEY WORDS

α-helix: a helical structure formed by a polypeptide chain, held in place by hydrogen bonds; an α-helix is an example of secondary structure in a protein

β-pleated sheet: a loose, sheet-like structure formed by hydrogen bonding between parallel polypeptide chains; a β-pleated sheet is an example of secondary structure in a protein

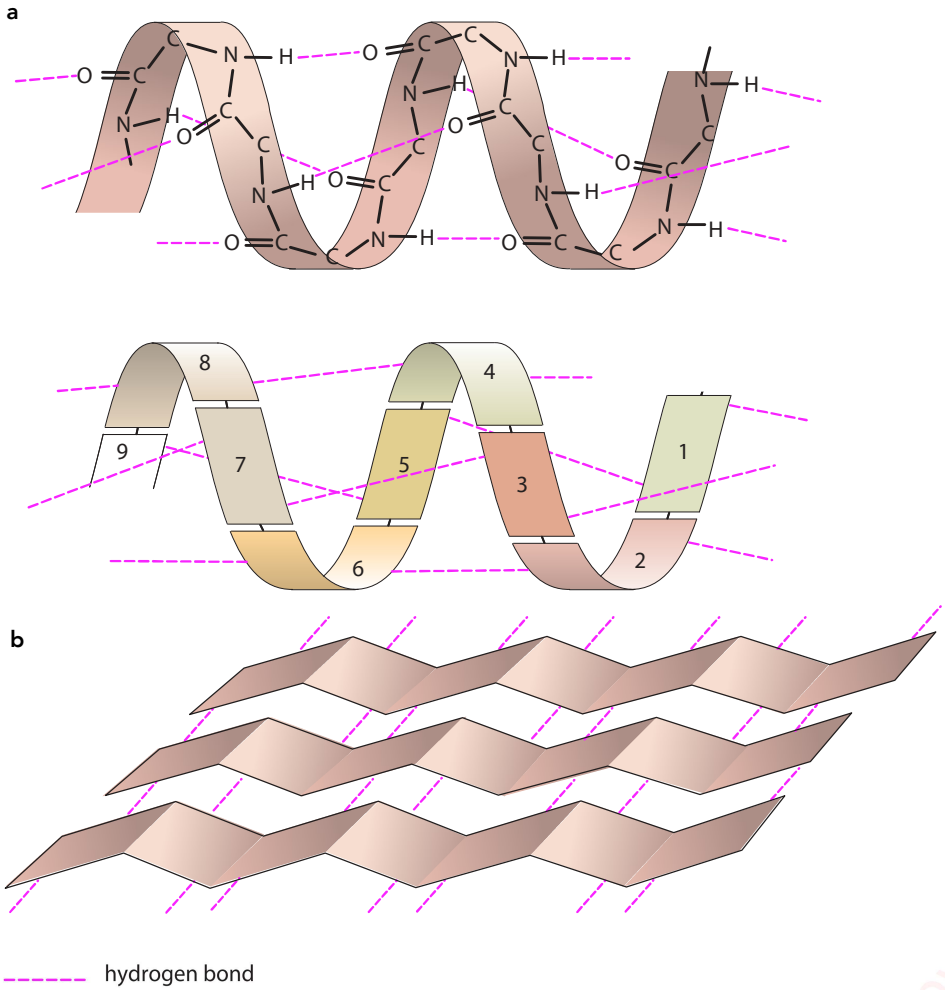


Figure 2.19: Protein secondary structure. **a** Structure of the α-helix. The R groups are not shown. **b** Another common secondary structure is the β-pleated sheet. Both of these structures are held in shape by hydrogen bonds between the amino acids.

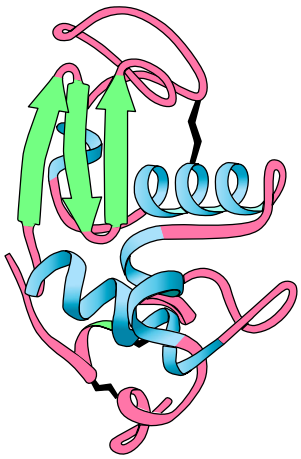


Figure 2.20: Secondary and tertiary structure of lysozyme. α-helices are shown as blue coils, β-pleated sheets as green arrows, and random coils as red ribbons. The black zig-zags are disulfide bonds.

Some proteins or parts of proteins show no regular arrangement at all. It all depends on which R groups are present and what attractions occur between amino acids in the chain.

In diagrams of protein structure, α-helices can be represented as coils or cylinders: β-pleated sheets as arrows, and random coils as ribbons (Figures 2.20 and 2.21).

Tertiary structure

In many proteins, the secondary structure itself is coiled or folded. Figures 2.20 and 2.21 show the complex way in which secondary structure coils or folds to form the **tertiary structure** of a protein. The figures show different ways in which the structures can be represented by diagrams. At first sight, the lysozyme and myoglobin molecules in these figures look like disorganised tangles, but this is not so. The shape of the molecules is very precise, and the molecules are held in these exact shapes by bonds between amino acids in different parts of the chain.

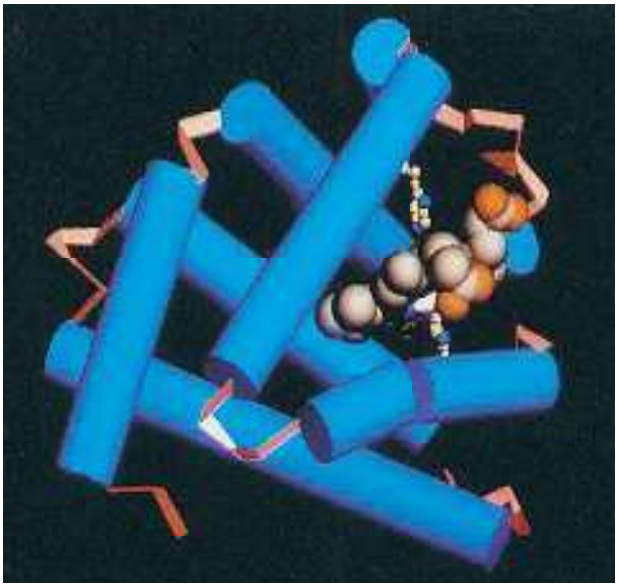


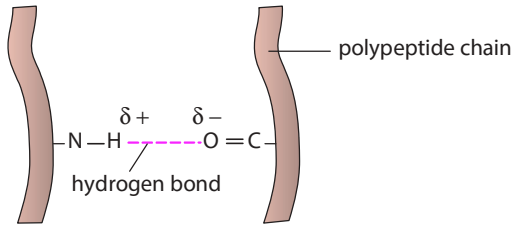
Figure 2.21: A computer graphic showing the secondary and tertiary structures of a myoglobin molecule. Myoglobin is the substance which makes meat look red. It is found in muscle, where it acts as an oxygen-storing molecule. The blue cylinders are α-helices and are linked by sections of polypeptide chain which are random coils (shown in red). At the centre right is an iron-containing haem group.

Figure 2.22 shows the four types of bond that help to keep folded proteins in their precise shapes.

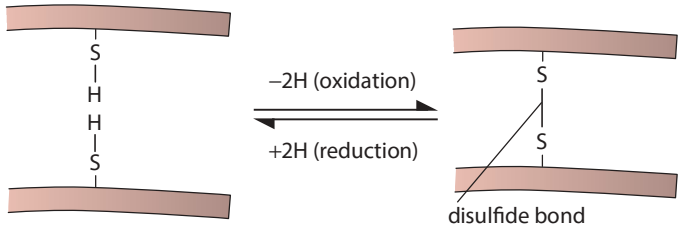
- Hydrogen bonds can form between a wide variety of R groups. Hydrogen bonds are weak in isolation but many together can form a strong structure.
- Disulfide bonds form between two cysteine molecules. Cysteine molecules contain sulfur atoms. The disulfide bond forms when the sulfur atoms of neighbouring cysteines join together with a covalent bond. This is a strong bond. (Can you spot the four disulfide bonds in ribonuclease in Figure 2.18?)
- Ionic bonds form between R groups containing amino and carboxyl groups. (Which amino acids have R groups containing amino or carboxyl groups?)
- Hydrophobic interactions occur between R groups that are non-polar. Such R groups are hydrophobic

possible. If the protein is in
nent inside the cell, then the
ill tend to come together,
rall shape of many proteins
phobic interactions.

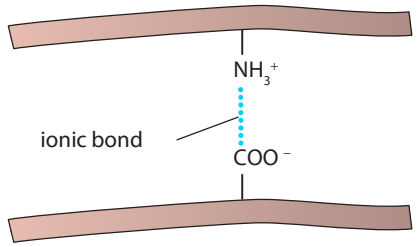
a **Hydrogen bonds** form between strongly polar groups – for example, –NH–, –CO– and –OH groups.



b **Disulfide bonds** form between cysteine molecules. They are strong covalent bonds. They can be broken by reducing agents.



c **Ionic bonds** form between ionised amino (NH_3^+) groups and ionised carboxylic acid (COO^-) groups. They can be broken by pH changes.



d **Weak hydrophobic interactions** occur between non-polar R groups. Although the interactions are weak, the groups tend to stay together because they are repelled by the watery environment around them.

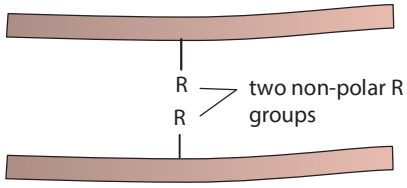


Figure 2.22: The four types of bond which are important in protein tertiary structure: **a** hydrogen bonds, which are also important in secondary structure; **b** disulfide bonds; **c** ionic bonds; **d** hydrophobic interactions.

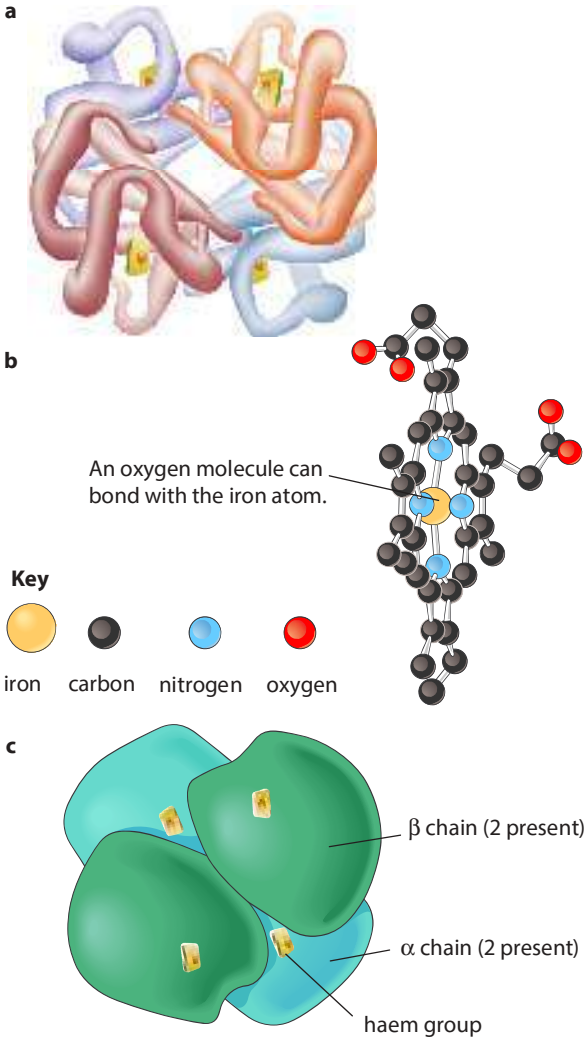


Figure 2.23: Haemoglobin. **a** Each haemoglobin molecule contains four polypeptide chains. The two α chains are shown in purple and blue, and the two β chains in brown and orange. Each polypeptide chain contains a haem group, shown in yellow and red. **b** The haem group contains an iron atom, which can bond reversibly with an oxygen molecule. **c** The complete haemoglobin molecule is nearly spherical.

The R groups are typically orientated towards the centre of the proteins, facing away from the outside watery environment, with the hydrophilic R groups surrounding them and pointing outwards and in contact with the watery environment.

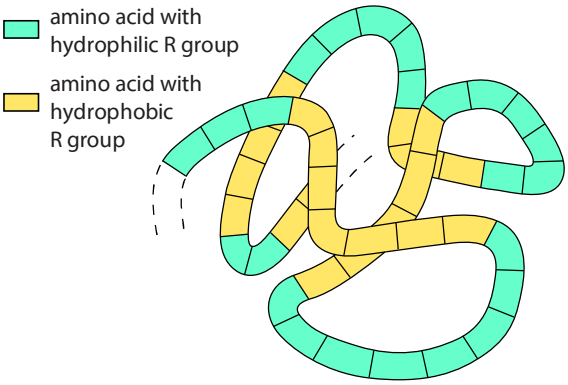
Quaternary structure

Many protein molecules are made up of two or more polypeptide chains. The overall structure formed by the different polypeptide chains is called the **quaternary structure** of the protein. **Haemoglobin** is an example of a protein with a quaternary structure. A molecule of haemoglobin has four polypeptide chains (Figure 2.23).

The polypeptide chains in quaternary structures are held together by the same four types of bond as in tertiary structures.

Globular and fibrous proteins

A protein whose molecules curl up into a ball shape, such as myoglobin or haemoglobin, is known as a **globular protein**. Globular proteins usually curl up so that their non-polar, hydrophobic R groups point into the centre of the molecule, away from their watery surroundings. Water molecules are excluded from the centre of the folded protein molecule. The polar, hydrophilic R groups remain on the outside of the molecule. Globular proteins, therefore, are usually soluble, because water molecules cluster around their outward-pointing hydrophilic R groups (Figure 2.24).



Many globular proteins have roles in metabolic reactions. Their precise shape is the key to their functioning. Enzymes, for example, are globular proteins.

Many other protein molecules do not curl up into a ball, but form long strands. These are known as fibrous proteins. Fibrous proteins are not usually soluble in water and most have structural roles. For example, the fibrous protein keratin forms hair, nails and the outer layers of skin, making these structures waterproof. Another example of a fibrous protein is collagen.

Haemoglobin – a globular protein

Haemoglobin is the oxygen-carrying pigment found in red blood cells. It is a globular protein. You have seen that it is made up of four polypeptide chains, so it has a quaternary structure. Each chain is a protein known as globin. Globin is related to myoglobin and so has a very similar tertiary structure (Figures 2.21 and 2.23). There are many types of globin – two types are used to make haemoglobin, and these are known as α-globin (alpha-globin) and β-globin (beta-globin). Two of the haemoglobin chains, called α chains, are made from α-globin, and the other two chains, called β chains, are made from β-globin.

The haemoglobin molecule is nearly spherical (Figure 2.23). The four polypeptide chains pack closely together. Their hydrophobic R groups point in towards the centre of the molecule, and their hydrophilic ones point outwards.

The interactions between the hydrophobic R groups inside the molecule are important in holding it in its correct three-dimensional shape. The outward-pointing hydrophilic R groups on the surface of the molecule are

KEY WORDS

quaternary structure: the three-dimensional arrangement of two or more polypeptides, or of a polypeptide and a non-protein component such as haem, in a protein molecule

haemoglobin: the red pigment found in red blood cells, whose molecules contain four iron atoms within a globular protein made up of four polypeptides; it combines reversibly with oxygen

in whose molecules are herical shape, often has often water-soluble and insulin, haemoglobin

important in maintaining its solubility. In the genetic condition known as **sickle cell anaemia**, one amino acid on the surface of the β chain is replaced with a different amino acid. The correct amino acid is glutamic acid, which is polar. The substitute is valine, which is non-polar. Having a non-polar R group on the outside of the molecule makes the haemoglobin much less soluble, and causes the unpleasant and dangerous symptoms associated with sickle cell anaemia (Figure 2.25).

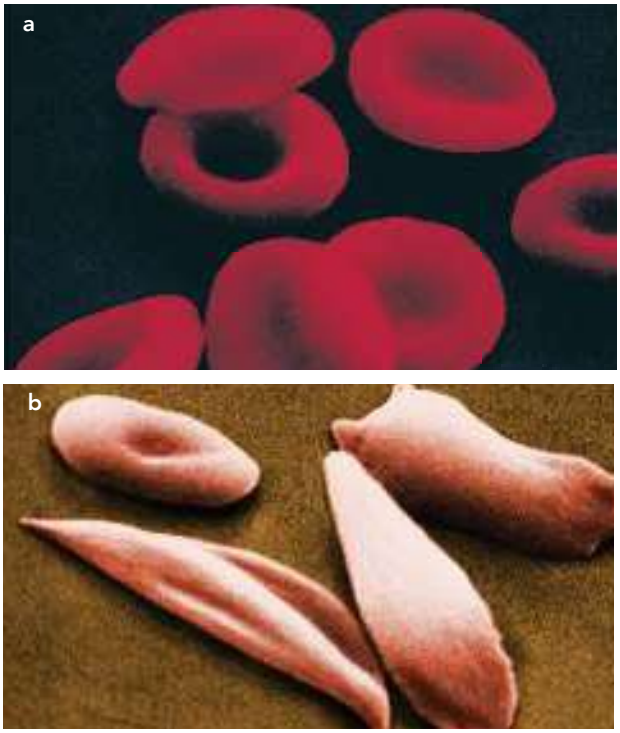


Figure 2.25: a Scanning electron micrograph of human red blood cells (×3300). Each cell contains about 250 million haemoglobin molecules. **b** Scanning electron micrograph of red blood cells from a person with sickle cell anaemia. You can see a normal cell and three sickled cells (×3300).

Each of the four polypeptide chains of haemoglobin contains a haem group (Figure 2.23b). A group like this, which is an important and permanent part of a protein molecule, but is not made of amino acids, is called a prosthetic group.

Each haem group contains an iron atom. One oxygen molecule (O_2) can bind with each iron atom. So a complete haemoglobin molecule, with four haem groups, can carry four oxygen molecules (eight oxygen atoms) at a time.

It is the haem group which is responsible for the colour of haemoglobin. This colour changes depending on whether or not the iron atoms are combined with oxygen. If they are, the molecule is known as oxyhaemoglobin and is bright red. If not, the colour is a darker, more bluish red.

Collagen – a fibrous protein

Collagen is the most common protein found in animals, making up 25% of the total protein in mammals. It is an insoluble **fibrous protein** (Figure 2.26) found in skin, tendons, cartilage, bones, teeth and the walls of blood vessels. It is an important structural protein in almost all animals.

KEY WORDS

sickle cell anaemia: a genetic disease caused by a faulty gene coding for haemoglobin, in which haemoglobin tends to precipitate when oxygen concentrations are low

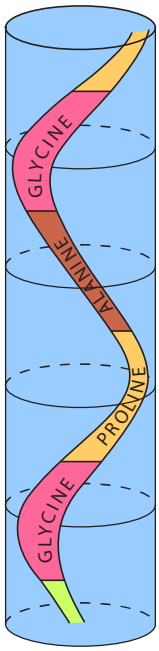
collagen: the main structural protein of animals; known as 'white fibres', the fundamental unit of the fibre consists of three helical polypeptide chains wound around each other, forming a 'triple helix' with high tensile strength

fibrous protein: a protein whose molecules have a relatively long, thin structure that is generally insoluble and metabolically inactive, and whose function is usually structural, e.g. keratin and collagen

A collagen molecule consists of three polypeptide chains, each in the shape of a helix (Figure 2.26b). (This is not an α-helix – it is not as tightly wound.) These three helical polypeptides are wound around each other, forming a three-stranded 'rope' or 'triple helix'. The three strands are held together by hydrogen bonds and some covalent bonds. Almost every third amino acid in each polypeptide is glycine, the smallest amino acid. Glycine is found on the insides of the strands and its small size allows the three strands to lie close together and so form a tight coil. Any other amino acid would be too large.

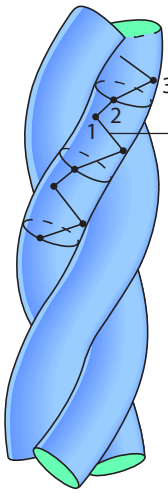
Each complete, three-stranded molecule of collagen interacts with other collagen molecules running parallel to it. Covalent bonds form between the R groups of amino acids lying next to each other. These cross-links hold many collagen molecules side by side, forming fibrils. The ends of the parallel molecules are staggered;

a



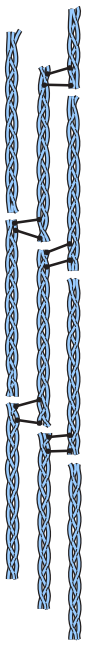
The polypeptides which make up a collagen molecule are in the shape of a stretched-out helix. Every third amino acid is glycine.

b



Three helices wind together to form a collagen molecule. These strands are held together by hydrogen bonds and some covalent bonds.

c



Many of these triple helices lie side by side, linked to each other by covalent cross-links between the side chains of amino acids near the ends of the polypeptides. Notice that these cross-links are out of step with each other; this gives collagen greater strength.

d

A scanning electron micrograph of collagen fibrils (× 17 000). Each fibril is made up of many triple helices lying parallel with one another. The banded appearance is caused by the regular way in which these helices are arranged, with the staggered gaps between the molecules (shown in **c**) appearing darker.

e

A scanning electron micrograph of human collagen fibres (×2000). Each fibre is made up of many fibrils lying side by side. These fibres are large enough to be seen with an ordinary light microscope.

the not-so-small. Thus, three
gen molecules make up a

if they were not, there would be a weak spot running right across the collagen fibril. Finally, many fibrils lie alongside each other, forming strong bundles called fibres. Note that many collagen molecules make up one collagen fibre.

The advantage of collagen is that it is flexible but at the same time has tremendous tensile strength. High tensile strength means it can withstand large pulling forces without stretching or breaking. The human Achilles tendon, which is almost pure collagen fibres, can withstand a pulling force of 300 N per mm^2 of cross-sectional area, about one-quarter the tensile strength of mild steel.

Collagen fibres are lined up in different structures according to the forces they must withstand. In tendons, they line up in parallel bundles along the length of the tendon, in the direction of tension. In skin, they may form layers, with the fibres running in different directions in the different layers, like cellulose in cell walls. In this way, they resist tensile (pulling) forces from many directions.

PRACTICAL ACTIVITY 2.4

Testing for the presence of proteins

All proteins have peptide bonds, containing nitrogen atoms. The nitrogen forms a purple complex with copper(II) ions and this forms the basis of the **biuret test**.

The reagent used for this test is called biuret reagent. You can use it as two separate solutions: a dilute solution of potassium hydroxide or sodium hydroxide, and a dilute solution of copper(II) sulfate. Alternatively, you can use a ready-made biuret reagent that contains both the copper(II) sulfate solution and the hydroxide ready mixed. To stop the copper ions reacting with the hydroxide ions and forming a precipitate, this ready-mixed reagent also contains sodium potassium tartrate or sodium citrate.

Procedure

The biuret reagent is added to the solution to be tested. No heating is required. A purple colour indicates that protein is present. The colour develops slowly over several minutes.

(See Practical Investigation 2.1 in the Practical Workbook for additional information.)

Questions

6 State **three** similarities and **three** differences between cellulose and collagen.

7 Copy Table 2.1. Fill in the blanks in the second column of the table using the words below:

hydrophilic haemoglobin ionic bond

hydrophobic disaccharide disulfide bond

Try with a partner to make a similar table with different statements based on the topics in this chapter. Try it out on other students.

8 'The protein-folding problem' box at the beginning of this chapter discussed how scientists are trying to predict the final shapes of proteins from a knowledge of their primary structures. What information about amino acids and proteins would be relevant to feed into a computer program trying to make such predictions?

KEY WORD

biuret test: a test for the presence of amine groups and thus for the presence of protein; biuret reagent is added to the unknown substance, and a change from pale blue to purple indicates the presence of protein

Description	Word/term
term for water-hating	
broken by a reduction reaction	
formed by a condensation reaction	
characteristic of globular proteins	
has two alpha chains and two beta chains	
can be broken by pH changes	

Table 2.1: Table for Question 7.

2.7 Water

Water is arguably the most important biochemical of all. Without water, life would not exist on this planet. It is important for two reasons. First, it is a major component of cells, typically forming between 70% and 95% of the mass of the cell. You are about 60% water. Second, it provides an environment for those organisms that live in water. Three-quarters of the planet is covered in water.

Although it is a simple molecule, water has some surprising properties. For example, such a small molecule would exist as a gas at normal Earth temperatures were it not for its special property of hydrogen bonding to other water molecules, discussed earlier. Also, because water is a liquid, it provides a medium for molecules and ions to mix in, and hence a medium in which life can evolve.

The hydrogen bonding of water molecules makes it more difficult to separate the molecules and thus affects the physical properties of water. For example, the energy needed to break the hydrogen bonds makes it relatively difficult to convert water from a liquid to a gas. It is more difficult than for similar compounds which lack hydrogen bonds, such as hydrogen sulfide (H_2S), which is a gas at normal air temperatures.

Water as a solvent

Water is an excellent solvent for ions and polar molecules (molecules with an uneven charge distribution, such as sugars and glycerol). This is because the water molecules are attracted to the ions and polar molecules and therefore collect around them and separate them (Figure 2.27). This is what happens when a chemical dissolves in water. Once a chemical is in solution, its molecules or ions are free to move about and react with other chemicals. Most processes in living organisms take place in solution in this way. The fact that molecules and ions dissolve in water also makes it ideal as a transport medium, for example, in the blood and lymphatic systems in animals, and in xylem and phloem in plants.

By contrast, non-polar molecules such as lipids are insoluble in water and, if surrounded by water, tend

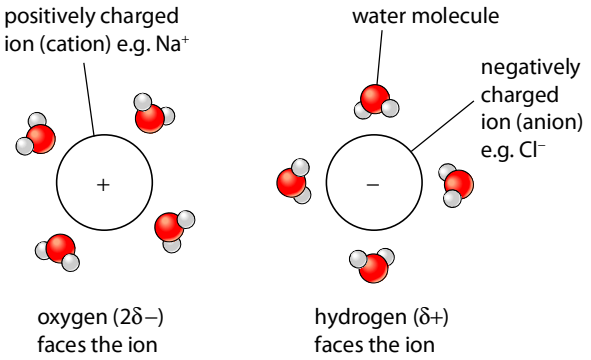


Figure 2.27: Distribution of water molecules around ions in a solution. Note which atoms of the water molecules face the ions.

High specific heat capacity

The heat capacity of a substance is the amount of heat required to raise its temperature by a given amount. The specific heat capacity of water is the amount of heat energy required to raise the temperature of 1 kg of water by 1 °C.

Water has a relatively high specific heat capacity. In order for the temperature of a liquid to be raised, the molecules must gain energy and consequently move about more rapidly. The hydrogen bonds that tend to make water molecules stick to each other make it more difficult for the molecules to move about freely. The bonds must be broken to allow free movement. This explains why more energy is needed to raise the temperature of water than would be the case if there were no hydrogen bonds. Hydrogen bonding, in effect, allows water to store more energy for a given temperature rise than would otherwise be possible.

The high specific heat capacity of water has important biological implications because it makes water more resistant to changes in temperature. This means that the temperature within cells and within the bodies of organisms (which have a high proportion of water) tends to be more constant than that of the air around them. As a result, biochemical reactions operate at relatively constant rates and are less likely to be adversely affected

[t also means that large
and oceans are slow to
nperature changes. As
vide stable habitats for

High latent heat of vaporisation

The latent heat of vaporisation is a measure of the heat energy needed to vaporise a liquid (cause it to evaporate), changing it from a liquid to a gas. In the case of water, it involves the change from liquid water to water vapour.

Water has a relatively high latent heat of vaporisation. This is a consequence of its high specific heat capacity. The fact that water molecules tend to stick to each other by hydrogen bonds means that relatively large amounts of energy are needed for vaporisation to occur, because hydrogen bonds have to be broken before molecules can escape as a gas. The energy transferred to water molecules during vaporisation results in a corresponding loss of energy from their surroundings, which therefore cool down. This is biologically important because it means that living organisms can use evaporation as a cooling mechanism, as in sweating or panting in mammals. A large amount of heat energy can be lost for relatively little loss of water, reducing the risk of dehydration. It can also be important in cooling leaves during transpiration.

The reverse is true when water changes from liquid to solid ice. This time the water molecules must lose a relatively large amount of energy, making it less likely that water will freeze. This is an advantage for aquatic organisms. It also makes it less likely that the bodies of living organisms, which have a high water content, will freeze.

Question

9 State the property of water that allows each of the following (**a**, **b** and **c**) to take place. Explain the importance of **a**, **b** and **c**:

- **a** the cooling of skin during sweating
- **b** the transport of glucose and ions in a mammal
- **c** much smaller temperature fluctuations in lakes and oceans than in terrestrial (land-based) habitats.

REFLECTION

- Explain the importance of simple biochemical molecules such as sugars, amino acids, organic bases, fatty acids and glycerol in the evolution of life.
- Water and carbon are important for life. How would you justify this statement?

Personal reflection questions

While studying this chapter, what activities have been particularly useful in improving your understanding of biochemistry? What does this show you about the way you like to learn?

Final reflection

Discuss with a friend which, if any, parts of Chapter 2 you need to:

- read through again to make sure you really understand
- seek more guidance on, even after going over it again.

SUMMARY

The larger biological molecules are made from smaller molecules. The smaller molecules are joined together by condensation reactions. Condensation involves removal of water. The reverse process, adding water, is called hydrolysis and is used to break the large molecules back down into smaller molecules. Polysaccharides are made from monosaccharides, proteins (polypeptides) from amino acids, lipids from fatty acids and glycerol. Polysaccharides and proteins are formed from repeating identical or similar subunits called monomers. They are, therefore, polymers. These build up into giant molecules called macromolecules.

Carbohydrates have the general formula $C_x(H_2O)_y$ and include monosaccharides, disaccharides and polysaccharides. Monosaccharides are joined together by glycosidic bonds to make disaccharides and polysaccharides. Monosaccharides (e.g. glucose) and disaccharides (e.g. sucrose) are very water-soluble and are known as sugars. They are important energy sources in cells and also important building blocks for larger molecules like polysaccharides.

Monosaccharides may have straight-chain or ring structures and may exist in different isomeric forms such as α-glucose and β-glucose.

Benedict's reagent can be used to test for reducing and non-reducing sugars. The test is semi-quantitative.

Polysaccharides include starch, glycogen and cellulose. Starch is an energy storage compound in plants. Starch is made up of two types of molecule, amylose and amylopectin, both made from α-glucose. Amylose is an unbranching molecule, whereas amylopectin has a branching structure.

'Iodine solution' can be used to test for starch.

Glycogen is an energy storage compound in animals. It is made from α-glucose. Its structure is similar to that of amylopectin but with more branching. Cellulose is a polymer of β-glucose molecules. The molecules are grouped together by hydrogen bonding to form mechanically strong fibres with high tensile strength that are found in plant cell walls.

Lipids are a diverse group of chemicals, the most common of which are triglycerides (fats and oils). Triglycerides are made by condensation between three fatty acid molecules and glycerol. Ester bonds join the fatty acids to the glycerol. Triglycerides are hydrophobic and do not mix with water. They act as energy storage compounds, as well as having other functions such as insulation and buoyancy in marine mammals. Phospholipids have a hydrophilic phosphate head and two hydrophobic fatty acid tails. This is important in the formation of membranes.

The emulsion test can be used to test for lipids.

Proteins are long chains of amino acids which fold into precise shapes. Amino acids are joined together by peptide bonds.

Proteins have up to four levels of structure known as primary, secondary, tertiary and quaternary structures. The primary structure is the sequence of amino acids in a protein. This largely determines the way that it folds and hence its three-dimensional shape and function.

Secondary structure is a result of hydrogen bonding between the amino acids. Examples of secondary structure are the α-helix and the β-pleated sheet. Further folding of proteins produces the tertiary structure. Often, a protein is made from more than one polypeptide chain. The association between the different chains is the quaternary structure of the protein. Tertiary and quaternary structures are very precise and are held in place by [illegible]ractions.

CONTINUED

Proteins may be globular or fibrous. A molecule of a globular protein – for example, haemoglobin – is roughly spherical. Most globular proteins are soluble and have physiological roles. Haemoglobin contains a non-protein (prosthetic) group, the haem group, which contains iron. This combines with oxygen. Molecules of a fibrous protein – for example, collagen – form long strands. Fibrous proteins are usually insoluble and have a structural role. Collagen has high tensile strength and is the most common animal protein, being found in a wide range of tissues.
Biuret reagent can be used to test for proteins.
Hydrogen bonding between water molecules gives water unusual properties.
Water is liquid at most temperatures on the Earth's surface. It has a high specific heat capacity, which makes liquid water relatively resistant to changes in temperature. Water acts as a solvent for ions and polar molecules, and causes non-polar molecules to group together. Water has a relatively high latent heat of vaporisation, meaning that evaporation has a strong cooling effect.

EXAM-STYLE QUESTIONS

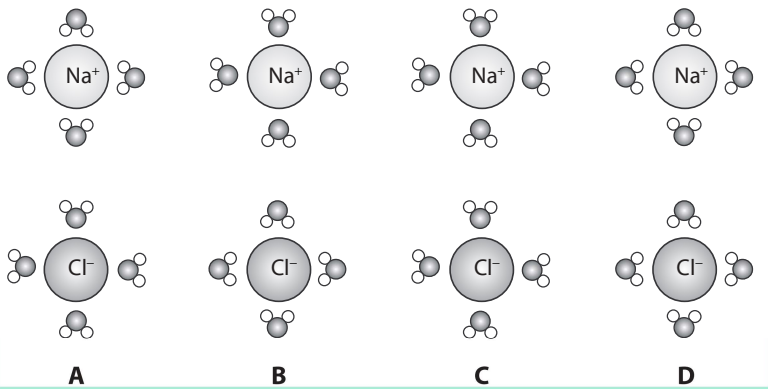
1 Which term describes both collagen and haemoglobin?

A enzymes
B fibrous proteins
C globular proteins
D macromolecules **[1]**

2 What type of chemical reaction is involved in the formation of disulfide bonds?

A condensation
B hydrolysis
C oxidation
D reduction **[1]**

3 Which diagram best represents the arrangement of water molecules around sodium (Na^+) and chloride (Cl^-) ions in solution? **[1]**



CONTINUED

4 Copy and complete the following table. Place a tick or a cross in each box as appropriate.

	Globular protein (e.g. haemoglobin)	Fibrous protein (e.g. collagen)	Monosaccharide	Disaccharide	Glycogen	Starch	Cellulose	Lipid
monomer								
polymer								
macromolecule								
polysaccharide								
contains subunits that form branched chains								
contains amino acids								
made from organic acids and glycerol								
contains glycosidic bonds								
contains peptide bonds								
one of its main functions is to act as an energy store								
usually insoluble in water								
usually has a structural function								
can form helical or partly helical structures								
contains only carbon, hydrogen and oxygen								

1 mark for each correct column **[8]**

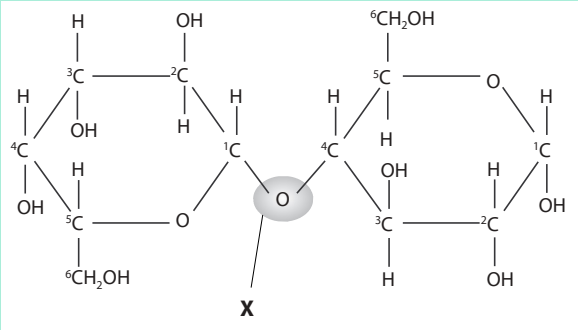
5 Copy the table and complete both columns. The table shows some functions of proteins, with examples of proteins that carry out these functions.

Function	Example
structural	1 2
enzyme	
	insulin

CONTINUED

6 State **three** characteristics of monosaccharides. **[3]**

7 The diagram shows a disaccharide called lactose. The carbon atoms are numbered. You are not expected to have seen this structure before. Lactose is a reducing sugar found in milk. It is made from a reaction between the two monosaccharides glucose and galactose.



a Suggest **two** functions that lactose could have. **[2]**

b What is the name given to the reaction between two monosaccharides that results in the formation of lactose? **[1]**

c Identify the bond labelled **X** in the diagram. **[1]**

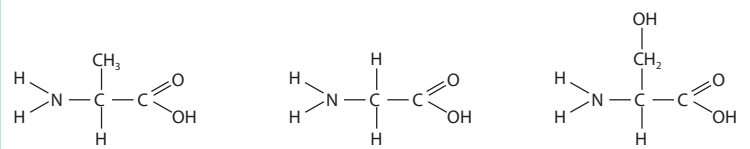
d Draw diagrams to show the structures of separate molecules of glucose and galactose. **[2]**

e Using the information in the diagram, is the alpha or beta form of glucose used to make lactose? **Explain** your answer. **[2]**

f Like lactose, sucrose is a disaccharide. If you were given a solution of lactose and a solution of sucrose, state briefly how you could distinguish between them. **[2]**

[Total: 10]

8 a This diagram shows the structures of three amino acids.



i Draw a diagram to show the structure of the tripeptide with the following sequence: alanine–glycine–serine. **[3]**

ii What is the name given to the sequence of amino acids in a protein? **[1]**

iii What substance, apart from the tripeptide, would be formed when the three amino acids combine? **[1]**

iv Draw a ring around an atom or group of atoms making up an R group that could hydrogen bond with a neighbouring R group. **[1]**

COMMAND WORD

Explain: set out purposes or reasons / make the relationships between things evident / provide why and/or how and support with relevant evidence.

CONTINUED

v Draw a ring around and label the peptide bond(s) you have drawn in the diagram. [1]

vi Draw a ring around a group of atoms which could hydrogen bond with a –CO– group in an alpha helix (α-helix). Label this group **A**. [1]

b State three features that α-helices and beta sheets (β-sheets) have in common. [3]

c A protein can be described as a polymer. State the meaning of the term *polymer*. [2]

d X and Y represent two different amino acids.

i Write down the sequences of all the possible tripeptides that could be made with just these two amino acids. [1]

ii From your answer to **d i**, what is the formula for calculating the number of different tripeptides that can be formed from two different amino acids? [1]

[Total: 15]

9 Copy diagrams A and B.

a Identify with labels which one represents a lipid and which a phospholipid. [1]

b i For molecule **A**, indicate on the diagram where hydrolysis would take place if the molecule was digested. [2]

ii Name the products of digestion. [2]

c Each molecule has a head with tails attached. For molecule **B**, label the head to identify its chemical nature. [1]

d i Which of the two molecules is water-soluble? [1]

ii Explain your answer to **d i**. [1]

e State **one** function of each molecule. [2]

[Total: 10]

10 a Copy and complete the table to summarise some differences between collagen and haemoglobin. [5]

	Collagen	Haemoglobin
1		
2		

CONTINUED

Use the following to guide you.

Row 1 State whether globular or fibrous.

Row 2 State whether entirely helical or partly helical.

Row 3 State the type of helix.

Row 4 State whether a prosthetic group is present or not.

Row 5 State whether soluble in water or not.

b State **one** way in which the structure of haemoglobin is related to its function. **[2]**

c Haemoglobin possesses a quaternary structure. What does this mean? **[1]**

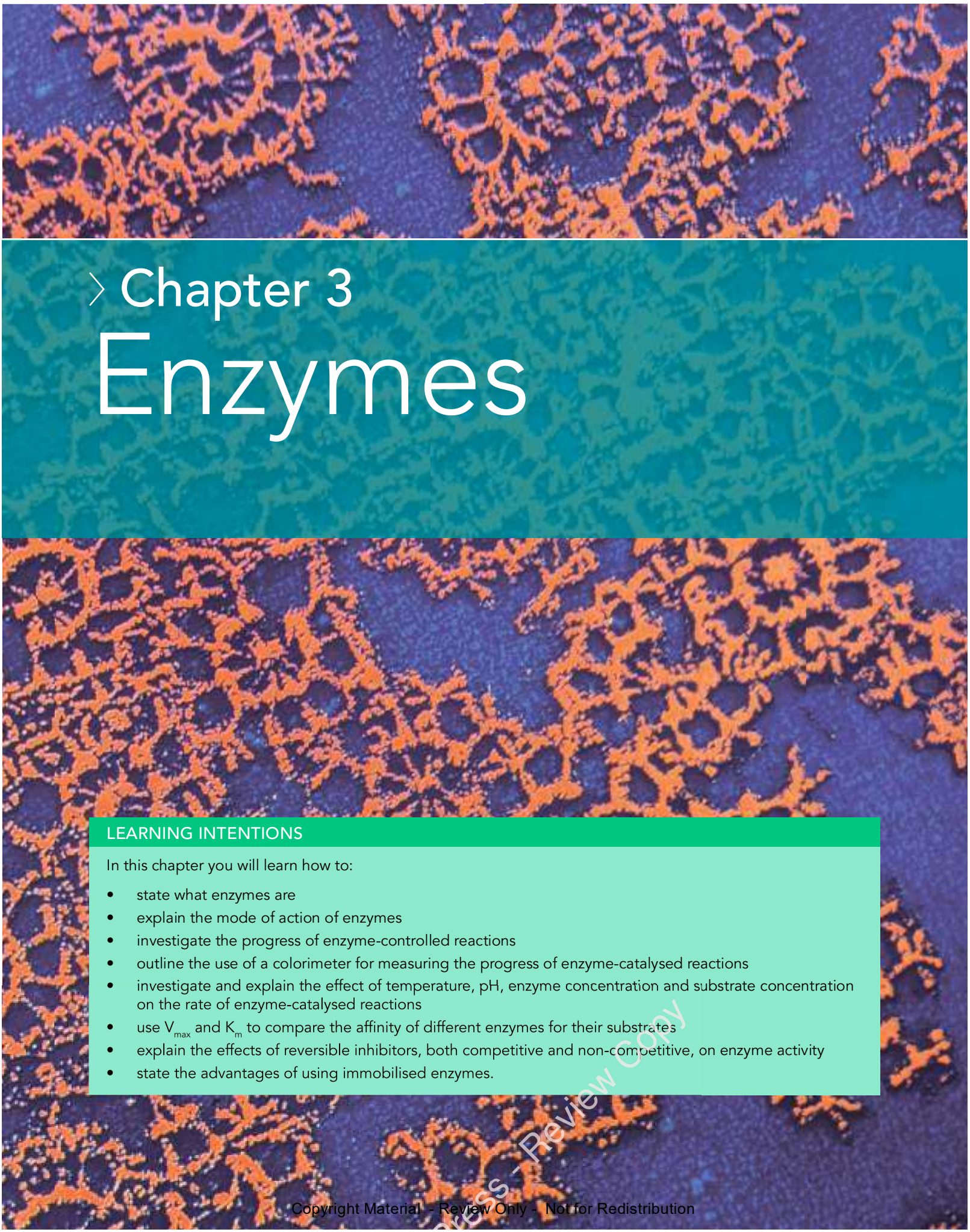
d Name the **five** elements found in haemoglobin. **[2]**

[Total: 10]

SELF-EVALUATION CHECKLIST

After studying this chapter, complete a table like this:

I can	See section...	Needs more work	Almost there	Ready to move on
describe how large biological molecules are made from smaller molecules	2.2, 2.3			
describe the structure of carbohydrates, lipids and proteins and how their structure relates to their functions	2.4, 2.5, 2.6			
describe and carry out biochemical tests to identify carbohydrates, lipids and proteins	2.4, 2.5, 2.6			
explain some key properties of water that make life possible	2.7			



Chapter 3
Enzymes

LEARNING INTENTIONS

In this chapter you will learn how to:

- state what enzymes are
- explain the mode of action of enzymes
- investigate the progress of enzyme-controlled reactions
- outline the use of a colorimeter for measuring the progress of enzyme-catalysed reactions
- investigate and explain the effect of temperature, pH, enzyme concentration and substrate concentration on the rate of enzyme-catalysed reactions
- use V_{max} and K_m to compare the affinity of different enzymes for their substrates
- explain the effects of reversible inhibitors, both competitive and non-competitive, on enzyme activity
- state the advantages of using immobilised enzymes.

BEFORE YOU START

- Enzymes are catalysts. Check your understanding of the term *catalyst* by stating two important properties of catalysts.
- Enzymes are proteins. You studied proteins in Chapter 2. Discuss what properties of proteins might make them suitable to act as catalysts in living cells.

THE BEST MEANS OF DEFENCE IS ATTACK

If you are a beetle and you are about to be eaten by a predator such as a spider or a frog, how do you escape? The bombardier beetle has evolved a spectacular and successful strategy (Figure 3.1). It makes use of the very high speeds of enzyme-controlled reactions. When threatened by a predator, the beetle uses the tip of its abdomen to squirt a boiling hot chemical spray at its attacker.

The release of the spray is accompanied by a loud popping sound. The beetle can swivel the tip of its abdomen to spray accurately in almost any direction. With the predator reeling from this surprise attack, the beetle escapes.

How are enzymes involved? Inside the beetle's abdomen is a chemical mixing chamber into which hydrogen peroxide and hydroquinone are released. The chamber contains two enzymes, catalase and peroxidase. These enzymes speed up the reactions they catalyse by several million times. Hydrogen peroxide is broken down into oxygen and water and the oxygen is used to oxidise the hydroquinone into quinone. The reactions are violent and release a great deal of heat, vaporising about 20% of the resulting liquid. Within a fraction of a second, a boiling, stinking mixture of gas and liquid is explosively released through an outlet valve.

Question for discussion

It could be argued that carrying out research into the defence mechanism of the bombadier beetle is a waste of time and money. Can you justify the research?

Figure 3.1: **a** A bombardier beetle sprays a boiling chemical spray at an annoying pair of forceps; **b** abdominal organs generating the spray.

3.1 What is an enzyme?

An **enzyme** is a biological catalyst. It is biological because all enzymes are proteins. It is a catalyst because it speeds up a chemical reaction but remains unchanged at the end of the reaction.

The following points are also important.

- Enzymes are globular proteins. They fold up into precise shapes.
- Almost all metabolic reactions which take place in living organisms are catalysed by enzymes; enzymes are therefore essential for life.
- Many enzyme names end in –ase; for example, amylase and ATPase.

Question

1 A student investigated the effect of several different catalysts on the rate of decomposition of hydrogen peroxide to water and oxygen. The speed of the reaction was judged by how 'fizzy' or frothy the contents of the tube became when the catalyst was added (oxygen is a product of the reaction and forms bubbles).

KEY WORD

enzyme: a protein produced by a living organism that acts as a biological catalyst in a chemical reaction by reducing activation energy

The student used iron filings and manganese dioxide as inorganic catalysts. They also used a commercial preparation of the enzyme catalase and pieces of liver and pieces of potato tuber, both of which contain catalase. Catalase catalyses the decomposition of hydrogen peroxide.

Results showed:

- catalase, liver and potato were much more efficient than the inorganic catalysts
- pure catalase was more efficient than the liver and potato
- liver was more efficient than potato
- ground-up liver was more efficient than pieces of liver.

Try to explain the student's results.

Intracellular and extracellular enzymes

Not all enzymes work inside cells. Those that do are described as intracellular. Enzymes that are secreted by cells and catalyse reactions outside cells are described as extracellular. Digestive enzymes in the gut are extracellular enzymes. Some organisms secrete enzymes outside their bodies. Fungi, for example, often do this in order to digest the food on which they are growing.

3.2 Mode of action of enzymes

The lock-and-key hypothesis and the induced-fit hypothesis

Like all globular proteins, enzyme molecules are coiled into a precise three-dimensional shape. Hydrophilic R groups (side-chains) on the outside of the molecule make them soluble in the water in the cytoplasm.

Enzyme molecules have a special feature called an **active site** (Figure 3.2). The active site of an enzyme is a region to which another molecule (or molecules) can bind. This molecule is the substrate of the enzyme. The shape of the active site allows the substrate to fit perfectly. The idea that the enzyme has a particular shape into which the substrate fits exactly is known as the **lock-and-key hypothesis**. The substrate is the key whose shape fits the lock of the enzyme. The substrate is held in place by temporary bonds which form between the substrate and some of the R groups of the enzyme's amino acids. This combined structure is termed the enzyme–substrate complex.

Each enzyme will act on only one type of substrate molecule. This is because the shape of the active site will only allow one shape of molecule to fit. The enzyme is said to be specific for this substrate. You can also describe the enzyme as showing specificity.

In 1959 the lock-and-key hypothesis was modified in the light of evidence that enzyme molecules are more flexible than is suggested by a rigid lock and key. The modern hypothesis for enzyme action is known as the **induced-fit hypothesis**. It is basically the same as the lock-and-key hypothesis, but adds the idea that the enzyme, and sometimes the substrate, can change shape slightly as the substrate molecule enters the enzyme, in order to ensure a perfect fit. This makes the catalysis even more efficient.

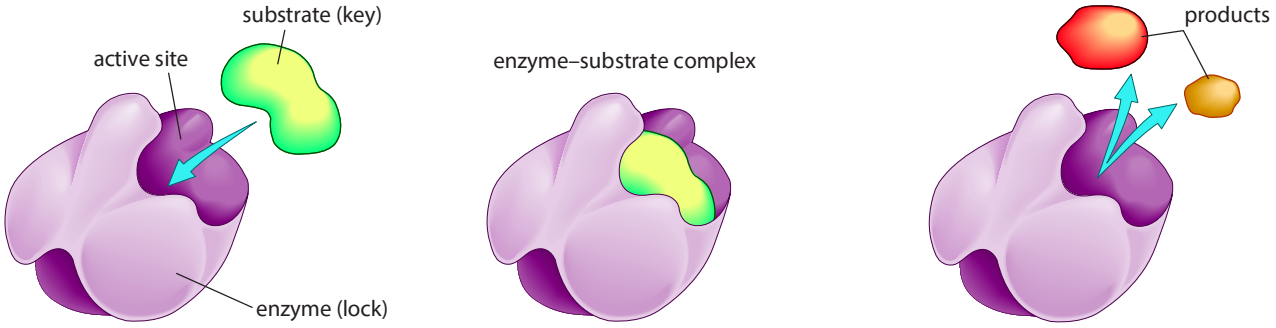
An enzyme may catalyse a reaction in which the substrate molecule is split into two or more molecules, as shown in Figure 3.2. Alternatively, it may catalyse the joining together of two molecules, as when making a dipeptide from two amino acids. A simplified diagram is shown in Figure 3.3. This diagram also shows the enzyme–product complex which is briefly formed before release of the product. When the reaction is complete, the product or products leave the active site. The enzyme is unchanged by this process, so it is now available to receive another substrate molecule.

KEY WORDS

active site: an area on an enzyme molecule where the substrate can bind

lock-and-key hypothesis: a hypothesis for enzyme action; the substrate is a complementary shape to the active site of the enzyme, and fits exactly into the site; the enzyme shows specificity for the substrate

induced-fit hypothesis: a hypothesis for enzyme action; the substrate is a complementary shape to the active site of the enzyme, but not an exact fit – the enzyme, or sometimes the substrate, can change shape slightly to ensure a perfect fit, but it is still described as showing specificity



a An enzyme has a cleft in its surface, called the active site. The substrate molecule has a complementary shape.

b Random movement of enzyme and substrate brings the substrate into the active site. An enzyme–substrate complex is temporarily formed. The R groups of the amino acids in the active site interact with the substrate.

c The interaction of the substrate with the active site breaks the substrate apart. An enzyme–product complex is briefly formed, before the two product molecules leave the active site, leaving the enzyme molecule unchanged and ready to bind with another substrate molecule.

Figure 3.2: How an enzyme catalyses the breakdown of a substrate molecule into two product molecules.

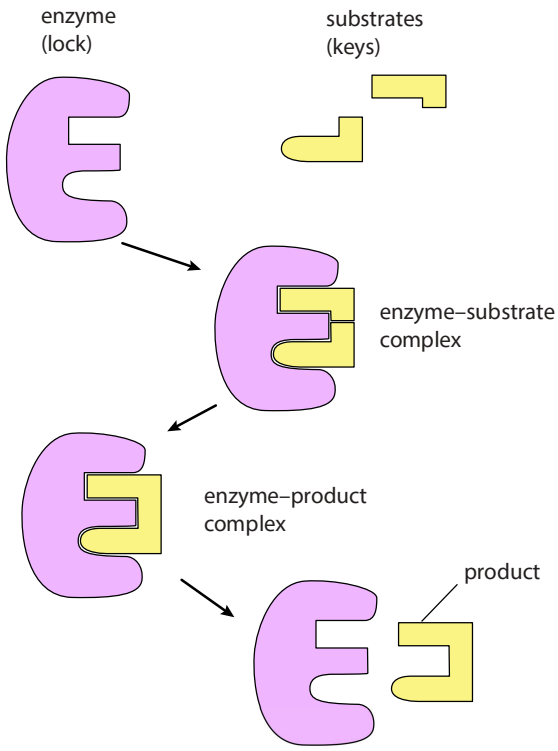


Figure 3.3: A simplified diagram of enzyme function. Note that in this example the enzyme is catalysing the joining together of two molecules.

The rate of the overall reaction can be very high. A molecule of the enzyme catalase, for example, can bind with hydrogen peroxide molecules, split them into water and oxygen, and release the products at a rate of 10 million molecules per second.

The example of lysozyme

The interaction between the substrate and the active site, including the slight change in shape of the enzyme (induced fit) which results from the binding of the substrate, is clearly shown by the enzyme lysozyme. Lysozyme is found in tears, saliva and other secretions. It acts as a natural defence against bacteria. It does this by breaking the polysaccharide chains that form the cell walls of the bacteria. The tertiary structure of the enzyme has already been shown in Figure 2.20. Figure 3.4 shows how the polysaccharide chains in the bacterial cell wall are broken down in the active site of lysozyme.

Enzymes reduce activation energy

Enzymes increase the rate at which chemical reactions occur. Without enzymes, most of the reactions that occur in living cells would occur so slowly that life could not exist.

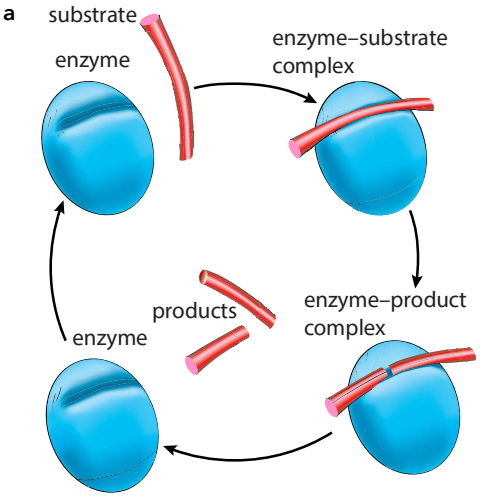


Figure 3.4: Lysozyme breaking a polysaccharide chain. This is a hydrolysis reaction. **a** Diagram showing the formation of enzyme–substrate and enzyme–product complexes, and release of the products. **b** Space-filling model showing the substrate in the active site of the enzyme. The substrate is a polysaccharide chain which slides neatly into the groove (active site) and is split by the enzyme. Many such chains give the bacterial cell wall rigidity. When the chains are broken, the wall loses its rigidity and the bacterial cell explodes as a result of osmosis.

In many chemical reactions, the substrate is not converted to a product unless some energy is added. This energy is called **activation energy** (Figure 3.5a).

One way of providing the extra energy needed is to heat the substances. For example, in the Benedict's test for reducing sugar you need to heat the Benedict's reagent and sugar solution together before they will react.

Enzymes avoid this problem because they decrease the activation energy of the reactions they catalyse (Figure 3.5b). They do this by holding the substrate or substrates in such a way that their molecules can react more easily. As a result, reactions catalysed by enzymes take place rapidly at a much lower temperature than they otherwise would.

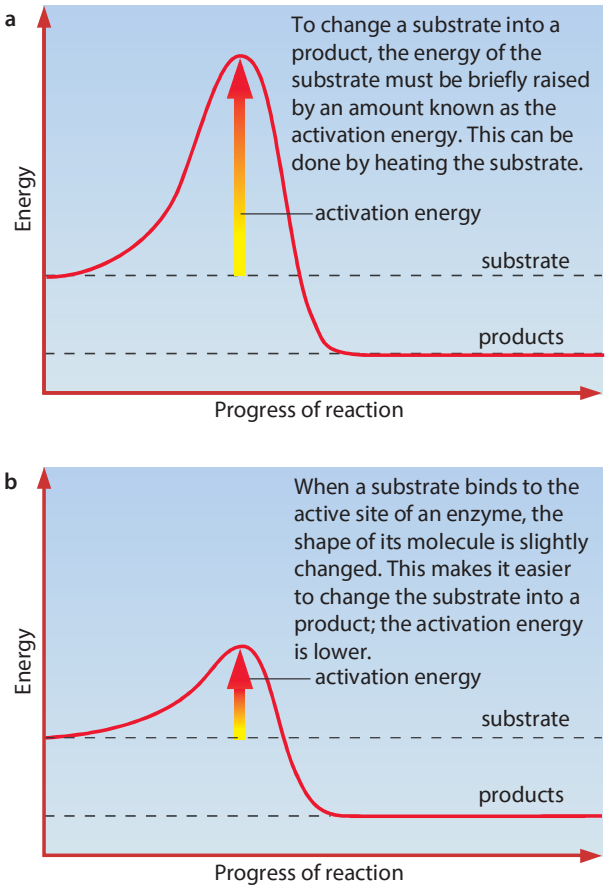


Figure 3.5: Activation energy: **a** without enzyme; **b** with enzyme.

KEY WORD

activation energy: the energy that must be provided to make a reaction take place; enzymes reduce the activation energy required for a substrate to change into a product

3.3 Investigating the progress of an enzyme-catalysed reaction

Measuring the rate of formation of a product

You may be able to carry out an investigation into the progress of an enzyme-controlled reaction by measuring the rate at which the product is formed from the substrate.

Figure 3.6 shows the results of such an investigation using the enzyme catalase. This enzyme is found in the tissues of most living things and catalyses the breakdown of hydrogen peroxide into water and oxygen. (Hydrogen peroxide is sometimes produced inside cells. It is toxic (poisonous), so it must be got rid of quickly.) The oxygen that is released can be collected and measured, so it is an easy reaction to follow.

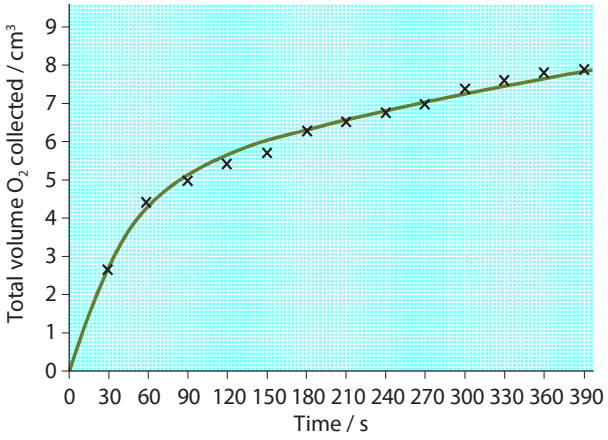


Figure 3.6: The course of an enzyme-catalysed reaction. Catalase was added to hydrogen peroxide at time 0. The gas released was collected in a gas syringe, the volume being read at 30s intervals.

The reaction begins very quickly. As soon as the enzyme and substrate are mixed, bubbles of oxygen are released. A large volume of oxygen is collected in the first minute of the reaction. As the reaction continues, however, the rate at which oxygen is released gradually slows down. The reaction gets slower and slower, until it eventually stops completely.

The explanation for the course of the reaction is quite straightforward. When the enzyme and substrate are first mixed, there are a large number of substrate molecules. At any moment, almost every enzyme molecule has a substrate molecule in its active site. The rate at which the reaction occurs depends on:

- how many enzyme molecules there are
- the speed at which the enzyme can convert the substrate into product, release it, and then bind with another substrate molecule.

However, as more and more substrate is converted into product, there are fewer and fewer substrate molecules to bind with enzymes. Enzyme molecules may be 'waiting' for substrate molecules to hit their active sites. As fewer and fewer substrate molecules are left, the reaction gets slower and slower, until it eventually stops.

The curve of a graph such as the one in Figure 3.6 is therefore steepest at the beginning of the reaction: the rate of an enzyme-controlled reaction is always fastest at the beginning. This rate is called the initial rate of reaction. You can measure the initial rate of the reaction by calculating the slope of a tangent to the curve, as close to time 0 as possible (see Figure P1.9 for advice on how to do this). An easier way of doing it is simply to read off the graph the amount of oxygen given off in the first 30 seconds. In this case, the rate of oxygen production in the first 30 seconds is 2.7 cm^3 of oxygen per 30 seconds, or 5.4 cm^3 per minute.

Question

2 Why is it better to calculate the initial rate of reaction from a curve such as the one in Figure 3.6 than simply by measuring how much oxygen is given off in 30 seconds?

Using a colorimeter to measure the progress of an enzyme-controlled reaction

If the method used for measuring the progress of an enzyme-controlled reaction involves a colour change, a **colorimeter** can be used to measure the colour change

KEY WORD

colorimeter: an instrument that measures the colour of a solution by measuring the absorption of different wavelengths of light

PRACTICAL ACTIVITY 3.1

Measuring the rate of disappearance of a substrate

Sometimes it is easier to measure the rate of disappearance of a substrate than the rate of appearance of a product. A good example of this is using the enzyme amylase. Amylase breaks down starch to the reducing sugar maltose, a hydrolysis reaction. Starch reacts with iodine solution to produce a blue-black colour. During the reaction, small samples can be taken at known times to test for starch using iodine solution. As the starch is converted to maltose, the concentration of starch in the reaction mixture decreases. The colour of the samples tested will, therefore, change from blue-black to brown to pale brown and finally remain colourless. You can time how long it takes for the starch to disappear completely, that is, how long before the iodine test gives a colourless result. Alternatively, a suitable end-point can be chosen, such as the time taken to reach a pale brown colour in the iodine test.

(See Practical Investigation 3.3 in the Practical Workbook for additional information.)

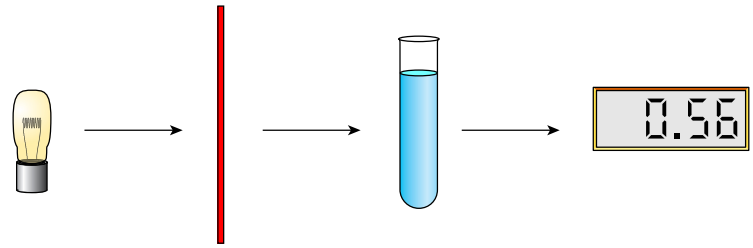
quantitatively. This will provide numbers that can be plotted on a graph. A colorimeter is an instrument that measures the colour of a solution by measuring the absorption of different wavelengths of light. The greater the absorption, the greater the concentration of the substance causing the colour. Figure 3.7a shows the main components of a colorimeter.

In the amylase/starch experiment described in Practical Activity 3.1 you can measure the intensity of the blue-black colour obtained in the iodine test using a colorimeter. The colour acts as a measure of the amount of starch still remaining. Figure 3.7b shows a typical range of colours.

If you do this over a period of time, you can plot a curve of 'amount of starch remaining' against 'time'. You can then calculate the initial reaction rate in the same way as for the catalase/hydrogen peroxide reaction described previously.

It is even easier to observe the course of this reaction if you mix starch, iodine solution and amylase in a tube, and take regular readings of the colour of the mixture in this one tube in a colorimeter. However, this is not ideal, because the iodine interferes with the rate of the reaction and slows it down.

a



b

Figure 3.7: a Diagram showing how a colorimeter works. b Photograph of a range of colours obtained with the iodine test during the course of an experiment investigating the digestion of starch by amylase. The tubes show increasing time for digestion of the starch from left to right.

Question

3 a In the breakdown of starch by amylase, if you were to plot the amount of starch remaining against time, draw the curve you would expect to obtain.

b How could you use this curve to calculate the initial reaction rate?

3.4 Factors that affect enzyme action

The effect of temperature on the rate of enzyme activity

Figure 3.8 shows the effect of temperature on the rate of activity of a typical enzyme. At low temperatures, the reaction takes place only very slowly. This is because molecules are moving relatively slowly. In other words, their kinetic energy is relatively low. Substrate molecules will not often collide with the active site of the enzyme. As temperature rises, the kinetic energy of the molecules increases and so the enzyme and substrate molecules move faster. Collisions happen more frequently, so that substrate molecules enter the active site more often. Also, when substrate and enzyme molecules collide, they do so with more energy. This makes it easier for bonds to be formed or broken so that the reaction can occur.

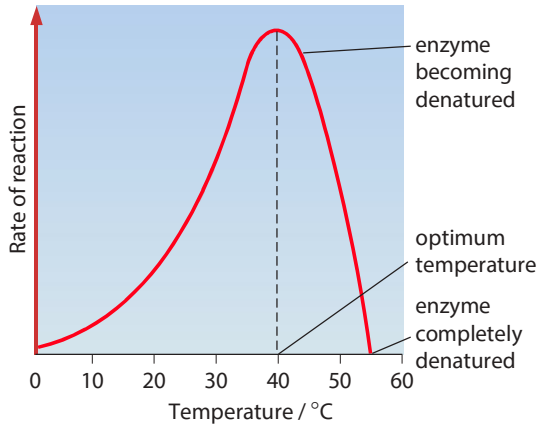


Figure 3.8: The effect of temperature on the rate of an enzyme-controlled reaction.

As temperature continues to increase, kinetic energy increases so the speed of movement of the substrate and enzyme molecules also continues to increase.

However, above a certain temperature, the enzyme molecule vibrates so much that some of the bonds holding the enzyme molecule in its precise shape begin to break. This is especially true for hydrogen bonds. The enzyme's active site begins to lose its shape and therefore its activity: it is said to be denatured. At first, the substrate molecule fits less well into the active site of the enzyme, so the rate of the reaction begins to slow down. Eventually the substrate no longer fits at all and the reaction stops (the rate becomes zero).

The temperature at which an enzyme catalyses a reaction at the maximum rate is called the optimum temperature. Most human enzymes have an optimum temperature of around 40 °C. By keeping our body temperatures at about 37 °C, we ensure that enzyme-catalysed reactions occur at close to their maximum rate.

Enzymes from other organisms may have different optimum temperatures. Some enzymes, such as those found in bacteria which live in hot springs (Figure 3.9), have much higher optimum temperatures. Some plant enzymes have lower optimum temperatures, depending on their habitat.

Figure 3.9: Not all enzymes have an optimum temperature of 40 °C. Bacteria and algae living in hot springs such as this one in Yellowstone National Park, USA, are able to tolerate very high temperatures. Enzymes from such organisms are useful in various commercial applications, such as biological washing powders.

The effect of pH on the rate of enzyme activity

Figure 3.10 shows the effect of pH on the rate of activity of a typical enzyme. Most enzymes work fastest at a pH of somewhere around 7, that is, in fairly neutral

conditions. Some, however, have a different optimum pH. For example, pepsin, an enzyme found in the acidic conditions of the stomach, has an optimum pH of about 1.5. Pepsin is a protease, an enzyme that catalyses the digestion of proteins.

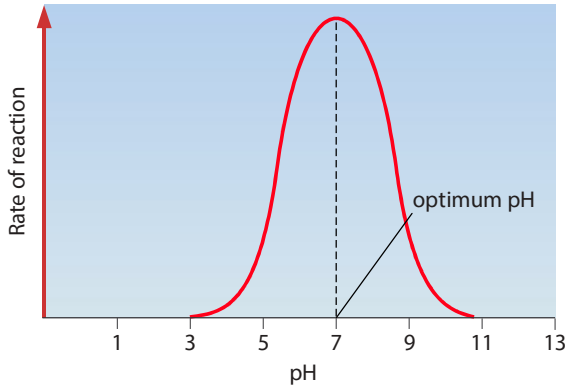


Figure 3.10: The effect of pH on the rate of an enzyme-controlled reaction. The optimum pH depends on the enzyme: in this case, the optimum pH is 7.

pH is a measure of the concentration of hydrogen ions in a solution. The lower the pH, the higher the hydrogen ion concentration. Hydrogen ions are positively charged, so they are attracted to negatively charged ions and repelled by positively charged ions. Hydrogen ions can therefore interact with any charged R groups on the amino acids of enzyme molecules. This may break the ionic bonding between the R groups (Chapter 2), which affects the three-dimensional structure of the enzyme molecule. The shape of the active site may change and therefore reduce the chances of the substrate molecule fitting into it. A pH which is different from the optimum pH can cause denaturation of an enzyme.

When investigating pH, you can use buffer solutions (Chapter P1, Section P1.3, Variables and making measurements). Buffer solutions each have a particular pH and maintain that pH even if the reaction taking place would otherwise cause the pH to change. You add a measured volume of the buffer to your reaction mixture.

Questions

4 How could you carry out an experiment to determine the effect of temperature on the rate of breakdown of hydrogen peroxide by catalase?

5 Proteases are used in biological washing powders.

a How does a protease remove a blood stain from clothes?

b Most biological washing powders are recommended for use at low washing temperatures. Why is this?

c Washing powder manufacturers have produced proteases which can work at temperatures higher than 40 °C. Why is this useful?

6 Trypsin is a protease secreted in pancreatic juice, which acts in the duodenum. If you add trypsin to a suspension of milk powder in water, the enzyme digests the protein in the milk, so that the suspension becomes clear.

How could you carry out an investigation into the effect of pH on the rate of activity of trypsin? (A suspension of 4 g of milk powder in 100 cm^3 of water will become clear in a few minutes if an equal volume of a 0.5% trypsin solution is added to it.)

The effect of enzyme concentration on the rate of enzyme activity

Figure 3.11a shows the results of an investigation using the enzyme catalase and its substrate hydrogen peroxide. The catalase is present in an extract made from celery. Different concentrations of catalase solution were added to hydrogen peroxide solutions. The different concentrations were prepared by varying the initial volume of celery extract and then making up to a standard volume with distilled water. The quantity of hydrogen peroxide (substrate) used was the same at the start of all five reactions.

You can see that the shape of all five curves is similar (Figure 3.11a). In each case, the reaction begins very quickly (a steep curve) and then gradually slows down (the curve levels off).

In order to look at the effect of enzyme concentration on reaction rate, you must compare the rates of these five reactions. It is best to look at the rate right at the beginning of the reaction. This is because, once the reaction is under way, the amount of substrate in each reaction begins to vary, because substrate is converted to product at different rates in each of the five reactions. It is only at the very beginning that you can be sure that the substrate concentration is the same

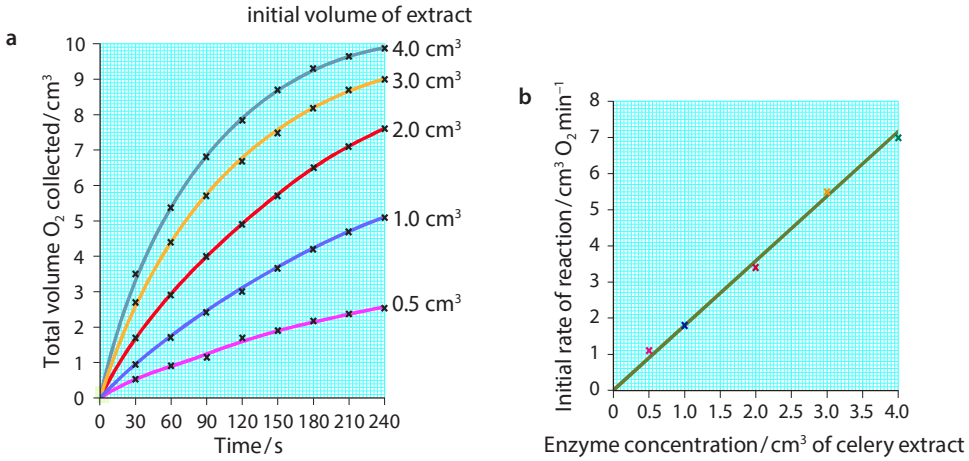


Figure 3.11: The effect of enzyme concentration on the rate of an enzyme-catalysed reaction. **a** Different volumes of celery extract, which contains catalase, were added to the same volume of hydrogen peroxide. Water was added to make the total volume of the mixture the same in each case. **b** The rate of reaction in the first 30s was calculated for each enzyme concentration.

in each tube. By calculating the initial rates you can be sure that differences in reaction rate are caused only by differences in enzyme concentration and not by substrate concentration.

To work out the initial rate for each enzyme concentration, you can calculate the slope of the curve 30 seconds after the beginning of the reaction, as explained in Chapter P1 (Section P1.3, Variables and making measurements). Ideally, you should do this for an even earlier stage of the reaction, but in practice this is impossible. You can then plot a second graph, Figure 3.11b, showing the initial rate of reaction against enzyme concentration.

This graph shows that the initial rate of reaction increases linearly. In these conditions, reaction rate is directly proportional to the enzyme concentration. This is just what common sense says should happen. The more enzyme present, the more active sites will be available for the substrate to slot into. As long as there is plenty of substrate available, the initial rate of a reaction increases linearly with enzyme concentration.

The effect of substrate concentration on the rate of enzyme activity

Figure 3.12 shows the results of an investigation using the enzyme catalase and its substrate hydrogen peroxide. The volume of hydrogen peroxide was varied and the volume of catalase was kept constant. As in the previous experiment, curves of oxygen released against time were plotted for each reaction, and the initial rate of reaction calculated for the first 30 seconds. These initial rates of reaction were then plotted against substrate concentration.

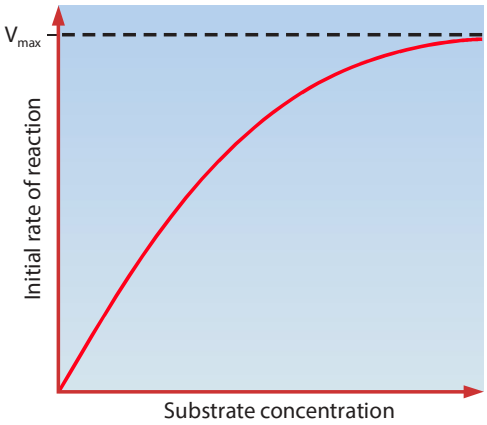


Figure 3.12: The effect of substrate concentration on the rate of an enzyme-catalysed reaction.

As substrate concentration increases, the initial rate of reaction also increases. This is what you would expect: the more substrate molecules there are, the more often one will enter an active site. However, if you go on increasing substrate concentration, keeping the enzyme concentration constant, there comes a point when every enzyme active site is full. If more substrate is added, the enzyme simply cannot work faster; substrate molecules

are effectively 'queuing up' for an active site to become vacant. The enzyme is working at its maximum possible rate, known as V_{max}. V stands for velocity (speed), max stands for maximum.

KEY WORD

V_{max}: the theoretical maximum rate of an enzyme-controlled reaction, obtained when all the active sites of the enzyme are occupied

Question

7 Sketch the shape that the graph in Figure 3.11b would have if excess hydrogen peroxide were not available.

3.5 Comparing enzyme affinities

Affinity is a measure of the strength of attraction between two things. A high affinity means there is a strong attraction. When applied to enzymes, affinity is a measure of the strength of attraction between the enzyme and its substrate. The greater the affinity of an enzyme for its substrate, the faster it works. Another way of thinking about this is to say that the higher the affinity, the more likely it is that the product will be formed when a substrate molecule enters the active site. If the affinity is low, the substrate may leave the active site before a reaction takes place.

There is enormous variation in the speed at which different enzymes work. A typical enzyme molecule can convert around 1000 substrate molecules into product per second. The enzyme carbonic anhydrase (Chapter 8, Section 8.5, Blood) is one of the fastest enzymes known. It can remove 600 000 molecules of carbon dioxide from respiring tissue per second. This is roughly 10^7 times as fast as the reaction would occur without the enzyme. It has presumably evolved such a high efficiency because a build-up of carbon dioxide in tissues would become lethal very quickly.

In the previous section, you saw that a useful indicator of the efficiency of an enzyme is its V_{max}. This tells you the maximum speed at which an enzyme works. Remember, V stands for velocity, which means speed.

At V_{max} all the enzyme molecules are bound to substrate molecules – the enzyme is saturated with substrate. All the active sites are full. V_{max} can be measured in the way described in Figure 3.11b. The initial rate of the reaction is measured at different substrate concentrations while keeping the enzyme concentration constant. As substrate concentration is increased, reaction rate rises until the reaction reaches its maximum rate, V_{max}.

Question

8 For each substrate concentration tested, the rate should be measured as soon as possible. Explain why.

The initial rate for each substrate concentration is plotted against substrate concentration, producing a curve like those shown in Figures 3.12 and 3.13. Unfortunately, this type of curve never completely flattens out in practice, as shown by the dashed line on both figures. It only does so in theory at infinite substrate concentration. You cannot, therefore, read off the value of V_{max} from the graphs in Figure 3.12 and 3.13. (Note that V_{max} is at the end of the dashed line in the figures.) There is a mathematical way out of this problem. From the data in such graphs, it is possible to calculate $\frac{1}{2}V_{max}$. You do not need to understand how to do this. $\frac{1}{2}V_{max}$ is exactly half the maximum velocity. It is just as useful as V_{max} as an indicator of how fast an enzyme works. You can plot $\frac{1}{2}V_{max}$ on a graph like Figures 3.12 and 3.13, and from that find the substrate concentration that will result in $\frac{1}{2}V_{max}$. This is the substrate concentration at which half the enzyme's active sites are occupied by substrate. Figure 3.13 shows a graph with $\frac{1}{2}V_{max}$ added. You will

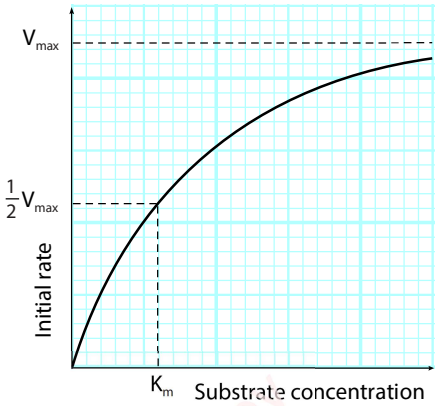


Figure 3.13: A graph showing the effect of substrate concentration on initial rate of an enzyme reaction, with V_{max}, $\frac{1}{2}V_{max}$ and K_m values shown.

Enzyme	Substrate	V_{max} / arbitrary units	K_m / μmol dm^{-3}
carbonic anhydrase	carbon dioxide	600000	8000
penicillinase	penicillin	2000	50
chymotrypsin	protein	100	5000
lysozyme	acetylglucosamine	0.5	6

Table 3.1: V_{max} and K_m values for four enzymes. Note that the unit for K_m is a concentration.

see that the substrate concentration which causes ½V_{max} is labelled K_m. **K_m** is known as the **Michaelis–Menten constant**. The Michaelis–Menten constant of an enzyme is the substrate concentration at which the enzyme works at half its maximum rate. It is used as a measure of the affinity of the enzyme for its substrate.

KEY WORD

Michaelis–Menten constant (K_m): the substrate concentration at which an enzyme works at half its maximum rate (½V_{max}), used as a measure of the efficiency of an enzyme; the lower the value of K_m, the more efficient the enzyme

If you think about it, the higher the affinity of an enzyme for its substrate, the lower the substrate concentration needed before ½V_{max} is reached. The lower the substrate concentration, the lower the value of K_m (see Figure 3.13). So the higher the affinity of an enzyme for its substrate, the lower its K_m will be.

Questions

9 Which of the four enzymes in Table 3.1:

- **a** works fastest?
- **b** has the highest affinity for its substrate? Briefly explain your answer.

10 Figure 3.14 shows the results of two experiments. The aim of the experiments was to compare the affinity of two different enzymes for their substrates. Enzyme **A** had a higher affinity for its substrate than enzyme **B**. The two curves plot the results obtained for enzyme **A** and enzyme **B**.

- **a** Copy Figure 3.14 and:
  - **i** label the axes appropriately
  - **ii** label appropriately one curve enzyme **A** and one curve enzyme **B**.
- **b**
  - **i** Which enzyme had the higher Michaelis–Menten constant?
  - **ii** Which enzyme had the higher V_{max}?
  - **iii** Which enzyme required the greater concentration of substrate to achieve V_{max}?
  - **iv** Which enzyme required the greater concentration of substrate to saturate its active sites?

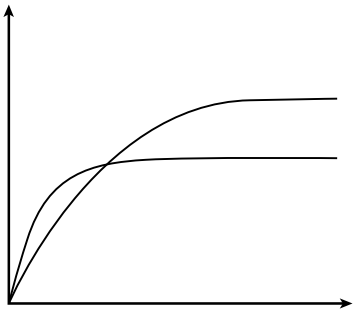


Figure 3.14: Comparison of affinity of two different enzymes for their substrates.

3.6 Enzyme inhibitors

Competitive, reversible inhibition

As you have seen, the active site of an enzyme fits its substrate perfectly. It is possible, however, for a molecule which is similar in shape to the substrate to enter an enzyme's active site. This would then inhibit the enzyme's function.

If an inhibitor molecule binds only briefly to the site and comes out again, there is competition between it and the substrate for the site. If there is much more of the substrate present than the inhibitor, substrate

molecules can easily 'win' the competition, and so the enzyme's function is more or less unaffected. However, if the concentration of the inhibitor rises or that of the substrate falls, it becomes less and less likely that the substrate will collide with an empty active site. The enzyme's function is then inhibited. This is known as **competitive inhibition** (Figure 3.15a). It is said to be reversible (not permanent) because it can be reversed by increasing the concentration of the substrate. This is how you can tell the difference between competitive and non-competitive inhibition (Figure 3.15).

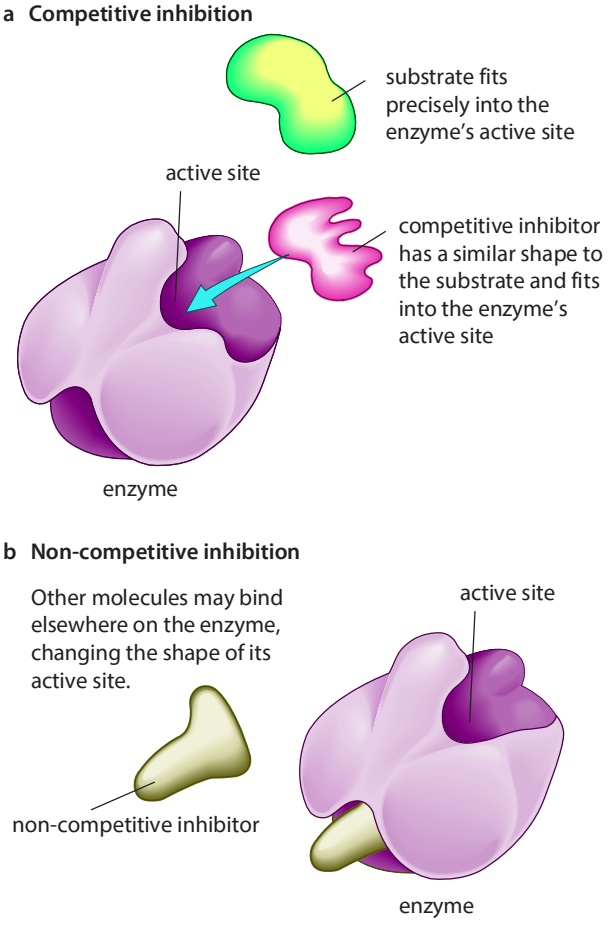


Figure 3.15: Enzyme inhibition: **a** competitive inhibition; **b** non-competitive inhibition. Both these types of inhibition are reversible.

An example of competitive inhibition occurs in the treatment of a person who has drunk ethylene glycol. Ethylene glycol is used as antifreeze, and is sometimes drunk accidentally. Ethylene glycol is rapidly converted in the body to oxalic acid, which can cause irreversible kidney damage. However, the active site of the enzyme which converts ethylene glycol to oxalic acid will also accept ethanol. If the poisoned person is given a large dose of ethanol, the ethanol acts as a competitive inhibitor, slowing down the action of the enzyme on ethylene glycol for long enough to allow the ethylene glycol to be excreted.

Non-competitive, reversible inhibition

A different kind of reversible inhibition is possible which is non-competitive. In this type of inhibition, the inhibitor molecule binds to another part of the enzyme, not the active site. While the inhibitor is bound to the enzyme, it can seriously affect the normal arrangement of hydrogen bonds and hydrophobic interactions holding the enzyme molecule in its three-dimensional shape. The resulting distortion changes the shape of the active site and therefore inhibits the ability of the substrate to enter the active site. While the inhibitor is attached to the enzyme, the enzyme's function is blocked. The substrate molecule and the inhibitor are not competing for the active site, so this is an example of **non-competitive inhibition** (Figure 3.15b). Increasing the substrate concentration has no effect on the inhibition, unlike the case with competitive inhibition.

Inhibition of an enzyme can be harmful or even fatal but, in many situations, inhibition is essential. For example, metabolic reactions must be controlled so that no enzyme can be allowed to work without stopping at some point, otherwise more and more product would constantly be being made.

KEY WORDS

competitive inhibition: when a substance reduces the rate of activity of an enzyme by competing with the substrate molecules for the enzyme's active site; increasing substrate concentration reduces the degree of inhibition; increasing inhibitor concentration increases the degree of inhibition

non-competitive inhibition: when a substance reduces the rate of activity of an enzyme, but increasing the concentration of the substrate does not reduce the degree of inhibition; many non-competitive inhibitors bind to areas of the enzyme molecule other than the active site itself

One way of controlling metabolic reactions is to use the end product of a chain of reactions as a non-competitive, reversible inhibitor (Figure 3.16). The end product inhibits the enzyme at the beginning of the chain of reactions (enzyme 1 in Figure 3.16). The enzyme is gradually slowed down as the amount of end product increases. However, the end product can lose its attachment to the enzyme (the reaction is reversible) so, if it gets used somewhere else, the enzyme returns to its active state and makes more end product. This way of regulating the amount of end product formed is called end product inhibition.

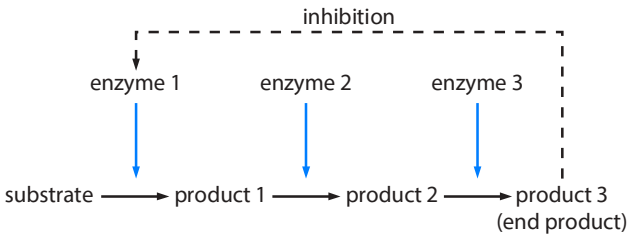


Figure 3.16: End product inhibition. As levels of product 3 rise, there is increasing inhibition of enzyme 1. So, less product 1 is made and therefore less product 2 and 3. Falling levels of product 3 allow increased function of enzyme 1 so products 1, 2 and 3 rise again and the cycle continues. This end product inhibition finely controls levels of product 3 between narrow upper and lower limits, and is an example of a feedback mechanism.

3.7 Immobilising enzymes

Enzymes have an enormous range of commercial applications – for example, in medicine, food technology and industrial processes. Enzymes are expensive. No company wants to have to keep buying them over and over again if it can recycle them in some way. One of the best ways of keeping costs down is to use **immobilised enzymes**. Immobilised enzymes are fixed in some way to prevent them from diffusing freely in a solution.

KEY WORD

immobilised enzymes: enzymes that have been fixed to a surface or trapped inside beads of agar gel

The enzyme lactase can be immobilised using alginate beads (Figure 3.17 in Practical Activity 3.2). The substrate of lactase is the disaccharide sugar lactose. Milk is allowed to run through a column of lactase-containing beads (Figure 3.18). The lactase hydrolyses the lactose in the milk to glucose and galactose. The milk is therefore lactose-free, and can be used to make lactose-free dairy products for people who cannot digest lactose.

You can see that enzyme immobilisation has several obvious advantages compared with just mixing up the enzyme with its substrate. If you just mixed lactase with milk, you would have a very difficult job to get the lactase back again. Not only would you lose the lactase, but you would also have milk contaminated with the enzyme. Using immobilised enzymes means that you can keep and re-use the enzymes, and that the product is enzyme-free.

Another advantage of this process is that the immobilised enzymes are more tolerant of temperature changes and pH changes than enzymes in solution. This may be partly because their molecules are held firmly in shape by the alginate in which they are embedded, and so do not denature so easily. It may also be because the parts of the molecules that are embedded in the beads are not fully exposed to the temperature or pH changes.

Questions

11 **a** Outline an investigation you could carry out to compare the temperature at which the enzyme lactase is completely denatured within ten minutes

i when free in solution

ii when immobilised in alginate beads.

b Outline an experiment you could carry out to investigate how long it takes the enzyme lactase to denature at 90 °C

i when free in solution

ii when immobilised in alginate beads.

c Outline how you would determine the optimum pH of the enzyme lactase

i when free in solution

ii when immobilised in alginate beads.

12 Summarise the advantages of using immobilised enzymes rather than enzyme solutions.

PRACTICAL ACTIVITY 3.2

Immobilising enzymes

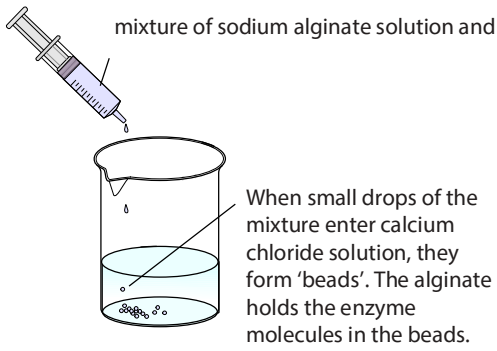


Figure 3.17: Immobilising enzyme in alginate.

Figure 3.17 shows one way in which enzymes can be immobilised. The enzyme is mixed with a solution of sodium alginate. Little droplets of this mixture are then added to a solution of calcium chloride. The sodium alginate and calcium chloride instantly react to form jelly, which turns each droplet into a little bead. The jelly bead contains the enzyme. The enzyme is held in the bead, or immobilised.

These beads can be packed gently into a column. A liquid containing the enzyme's substrate can be allowed to trickle steadily over them (Figure 3.18).

As the substrate runs over the surface of the beads, the enzymes in the beads catalyse a reaction that converts the substrate into product. The product continues to trickle down the column, emerging from the bottom, where it can be collected and purified.

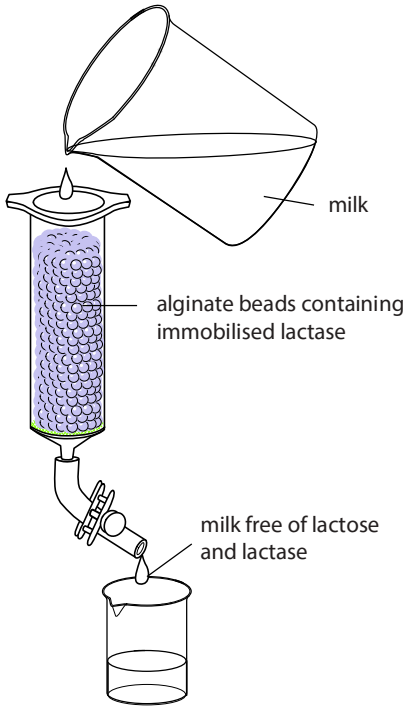


Figure 3.18: Using immobilised enzyme to modify milk.

(See Practical Investigation 3.5 in the Practical Workbook for additional information.)

REFLECTION

If you were designing a new enzyme to solve a complex biological or chemical problem, what characteristics and features would it be useful to be able to control? Try to think of both structural and functional features. Can you think of any new uses for enzymes, such as substances it would be useful to be able to break down using enzymes?

Personal reflection question

If you were the teacher, what comments would you make about your performance in this activity?

Final reflection

Discuss with a friend which, if any, parts of Chapter 3 you need to:

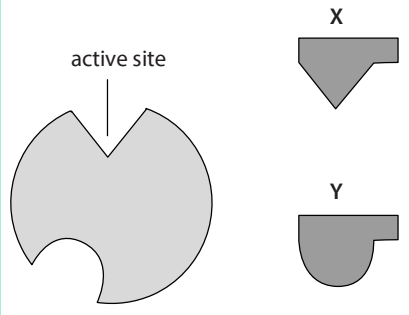
- read through again to make sure you really understand
- seek more guidance on, even after going over it again.

SUMMARY

Enzymes are globular proteins which catalyse metabolic reactions. Each enzyme has an active site with a flexible structure which can change shape slightly to fit precisely the substrate molecule. This is called the induced-fit hypothesis.
Enzymes may be involved in reactions which break down molecules or join molecules together. They work by lowering the activation energy of the reactions they catalyse.
The course of an enzyme reaction can be followed by measuring the rate at which a product is formed or the rate at which a substrate disappears. A progress curve, with time on the *x*-axis, can be plotted. The curve is steepest at the beginning of the reaction, when substrate concentration is at its highest. This rate is called the initial rate of reaction.
Temperature, pH, enzyme concentration and substrate concentration all affect the rate of activity of enzymes.
Each enzyme has an optimum temperature at which it works fastest. As temperature increases above the optimum temperature, the enzyme gradually denatures (loses its precise tertiary structure).
Each enzyme has an optimum pH. Some enzymes operate within a narrow pH range; some have a broad pH range.
The greater the concentration of the enzyme, the faster the rate of reaction, provided there are enough substrate molecules present. The greater the concentration of the substrate, the faster the rate of reaction, provided enough enzyme molecules are present. During enzyme reactions, rates slow down as substrate molecules are used up.
The efficiency of an enzyme can be measured by finding the value known as the Michaelis–Menten constant, K_m. To do this, the maximum rate of reaction, V_{max}, must first be determined. Determination of V_{max} involves finding the initial rates of reactions at different substrate concentrations while ensuring that enzyme concentration remains constant.
Enzymes are affected by the presence of inhibitors, which slow down or stop their activity. Competitive inhibitors have a similar shape to the normal substrate molecules. They compete with the substrate for the active site of the enzyme. Competitive inhibition is reversible because the inhibitor can enter and leave the active site.
Reversible non-competitive inhibitors bind at a site elsewhere on the enzyme, causing a change in shape of the active site.
Enzymes can be immobilised – for example, by trapping them in jelly (alginate) beads. This is commercially useful because the enzyme can be re-used and the product is separate from (uncontaminated by) the enzyme. Immobilisation often makes enzymes more stable.

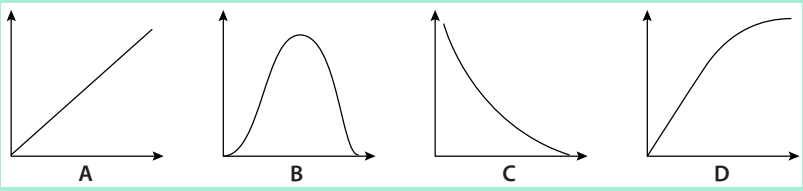
EXAM-STYLE QUESTIONS

1 The diagram shows an enzyme and two inhibitors of the enzyme, **X** and **Y**. Which of the following describes the two inhibitors?



A **X** and **Y** are competitive inhibitors.
B **X** and **Y** are non-competitive inhibitors.
C **X** is a competitive inhibitor and **Y** is a non-competitive inhibitor.
D **X** is a non-competitive inhibitor and **Y** is a competitive inhibitor. **[1]**

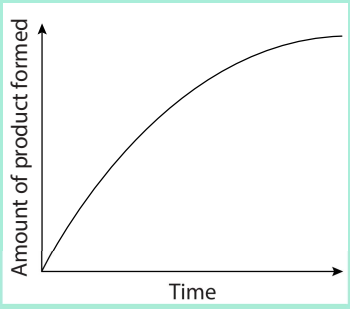
2 In a reaction controlled by an enzyme, which of the following graphs shows the effect of substrate concentration on the rate of the reaction, assuming the reaction is allowed to go to completion?



[1]

3 The graph shows the progress of the digestion of starch by the enzyme salivary amylase. Why does the reaction slow down?

A There is end-product inhibition by maltose.
B The salivary amylase is becoming denatured.
C The salivary amylase is gradually becoming saturated with starch.
D There are fewer and fewer substrate molecules left to bind with the salivary amylase.



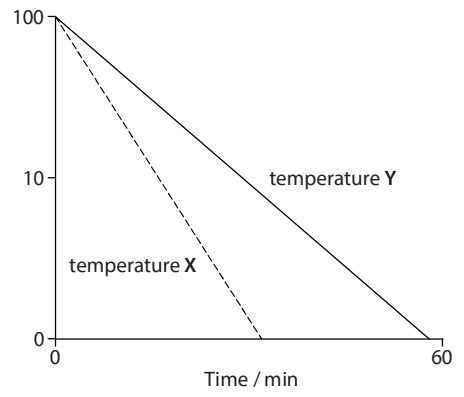
[1]

4 An enzyme with a high affinity for its substrate has a:

A high K_m
B low K_m
C high V_{max}
D low V_{max} **[1]**

CONTINUED

5 If methylene blue dye is added to a suspension of yeast cells, any living cells remain colourless. However, any dead cells are stained blue. This fact was used to carry out an investigation into the rate at which yeast cells were killed at two different temperatures (at high temperatures the yeast enzymes will be denatured). The results are shown in the diagram.



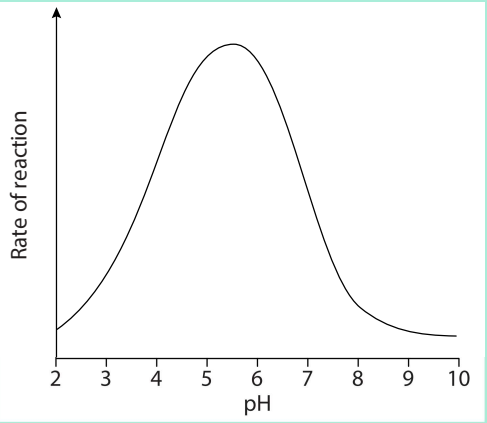
Which of the following is correct?

	The higher temperature is	The vertical axis (y-axis) should be labelled
A	X	% coloured cells
B	Y	% coloured cells
C	X	% colourless cells
D	Y	% colourless cells

[1]

6 Copy the graph in Question **3** and draw a line from which the initial rate of reaction could be calculated. **[1]**

7 The graph shows the effect of changes in pH on the activity of the enzyme lysozyme.



a Describe the effect of pH on this enzyme. **[2]**

b Explain why pH affects the activity of the enzyme. **[4]**

[Total: 6]

CONTINUED

8 The graph shows the effect of temperature on the rate of reaction of an enzyme.

a What is indicated by **X**? **[1]**

b What temperature would **X** be for a mammalian enzyme? **[1]**

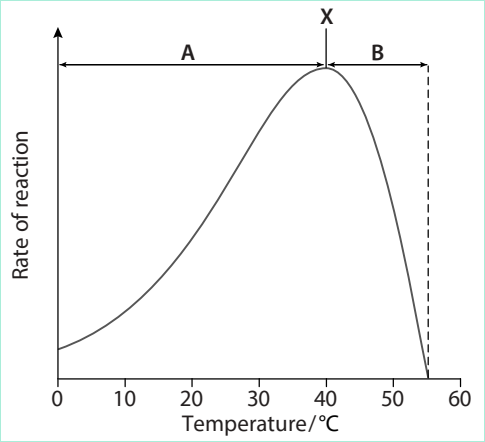
c Explain what is happening in region **A**. **[3]**

d Explain what is happening in region **B**. **[3]**

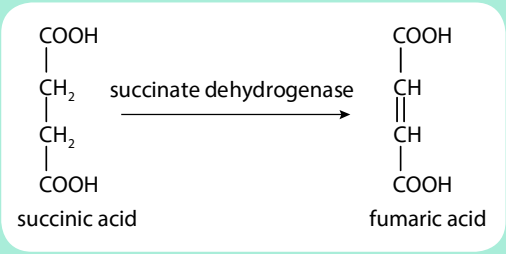
e Enzymes are effective because they lower the activation energy of the reactions they catalyse.

Explain what is meant by 'activation energy'. **[2]**

[Total: 10]

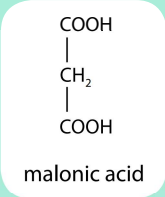


9 The reaction shown occurs during aerobic respiration. The reaction is catalysed by the enzyme succinate dehydrogenase.



a Name the substrate in this reaction. **[1]**

b The molecule malonic acid, which is shown below, inhibits the reaction shown. It does not bind permanently to the enzyme. Describe how malonic acid inhibits the enzyme succinate dehydrogenase. **[3]**



CONTINUED

c Heavy metals such as lead and mercury bind permanently to –SH groups of amino acids present in enzymes. These –SH groups could be in the active site or elsewhere in the enzyme.

i Name the amino acid which contains –SH groups. **[1]**

ii Explain the function of –SH groups in proteins and why binding of heavy metals to these groups might inhibit the activity of an enzyme. **[4]**

[Total: 9]

10 You are provided with three solutions, **A**, **B** and **C**. One solution contains the enzyme amylase, one contains starch and one contains glucose. Starch is the substrate of the enzyme. The product is the sugar maltose. You are provided with only one reagent, Benedict's solution, and the usual laboratory apparatus.

a **Outline** the procedure you would follow to identify the three solutions. **[6]**

b What type of reaction is catalysed by the enzyme? **[1]**

[Total: 7]

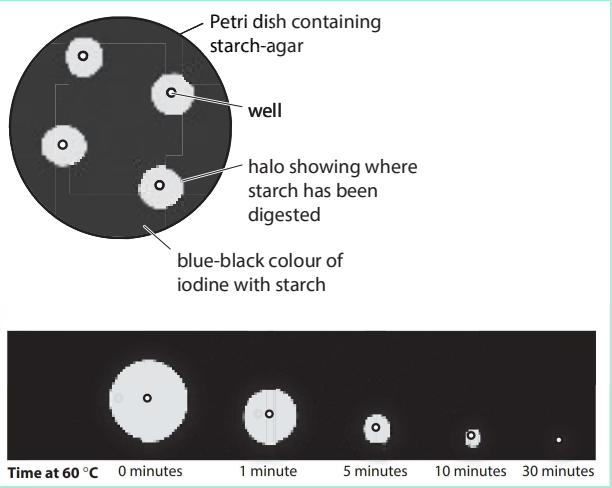
COMMAND WORD

Outline: set out the main points.

11 The activity of the enzyme amylase can be measured at a particular temperature by placing a sample into a Petri dish containing starch–agar ('a starch–agar plate'). Starch–agar is a jelly containing starch. One or more 'wells' (small holes) are cut in the agar jelly with a cork borer, and a sample of the enzyme is placed in each well. The enzyme molecules then diffuse through the agar and gradually digest any starch in their path. At the end of the experiment, iodine solution is poured over the plate. Most of the plate will turn blue-black as iodine reacts with starch, but a clear 'halo' (circle) will be seen around the well where starch has been digested. Measuring the size of the halo can give an indication of the activity of the enzyme.

A student decided to investigate the rate at which a mammalian amylase is denatured at 60 °C. They heated different samples of the enzyme in a water bath at 60 °C for 0, 1, 5, 10 and 30 minutes. They then allowed the samples to cool down to room temperature and placed samples of equal volume in the wells of five starch–agar plates, one plate for each heating period. They then incubated the plates in an oven at 40 °C for 24 hours.

The results of the student's experiment are shown below. A diagram of one dish is shown, and the real size of one halo from each dish is also shown.



CONTINUED

a Why did the student cut four wells in each dish rather than just one? **[1]**

b One dish contained samples from amylase which was not heated (0 minutes). This is a control dish.

Explain the purpose of this control. **[1]**

c Explain why the starch–agar plates were incubated at 40 °C and not room temperature. **[1]**

d Describe what was happening in the dishes during the 24 hours of incubation. **[4]**

e Why was it important to add the same volume of amylase solution to each well? **[1]**

f Measure the diameter in mm of the representative halo from each dish. Record the results in a suitable table. **[4]**

g Only one halo from each dish is shown in the diagrams. In practice, there was some variation in the diameters of the four halos in each dish. How would you allow for this when processing your data? **[1]**

h Plot a graph to show the effect of length of time at 60 °C on the activity of the enzyme. **[5]**

i Describe and explain your results. **[4]**

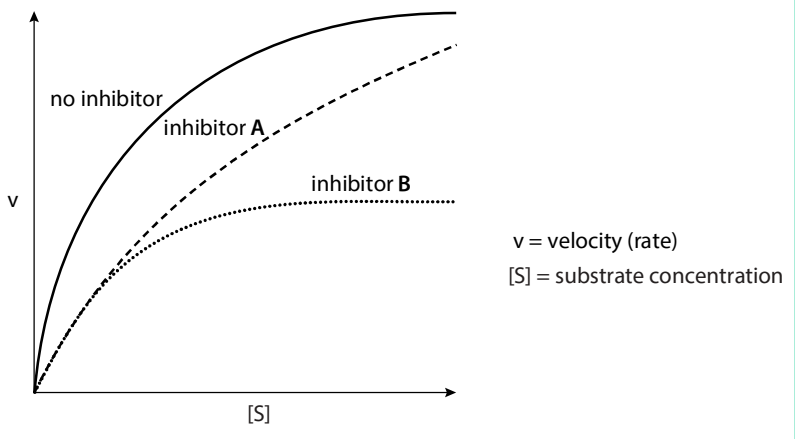
j Another student discovered that amylases from fungi and bacteria are more resistant to high temperatures than mammalian amylases. Using starch–agar plates as a method for measuring the activity of an amylase at 40 °C, outline an experiment that the student could perform to discover which type of amylase (mammalian, fungal or bacterial) is most resistant to heat. Note that temperatures up to 120 °C can be obtained by using an autoclave (pressure cooker). **[5]**

k Enzymes are used in many industrial processes where resistance to high temperatures is an advantage. State **three** other variables apart from temperature which should be controlled in an industrial process involving enzymes. **[3]**

[Total: 30]

12 Two inhibitors of the same enzyme, inhibitor **A** and inhibitor **B**, were investigated to discover if they were competitive or non-competitive. In order to do this, the rate of reaction of the enzyme was measured at different concentrations of substrate without inhibitor, with inhibitor **A** and with inhibitor **B**. Graphs of the data were plotted as shown. The graphs showed that one inhibitor was competitive and the other non-competitive.

CONTINUED



Copy the graphs.

a Label the graph for 'no inhibitor' to show the position of V_{max}, $\frac{1}{2}V_{max}$ and K_m. **[3]**

b State the effect that inhibitor **A** had on V_{max} and K_m of the enzyme. **[2]**

c State the effect that inhibitor **B** had on V_{max} and K_m of the enzyme. **[2]**

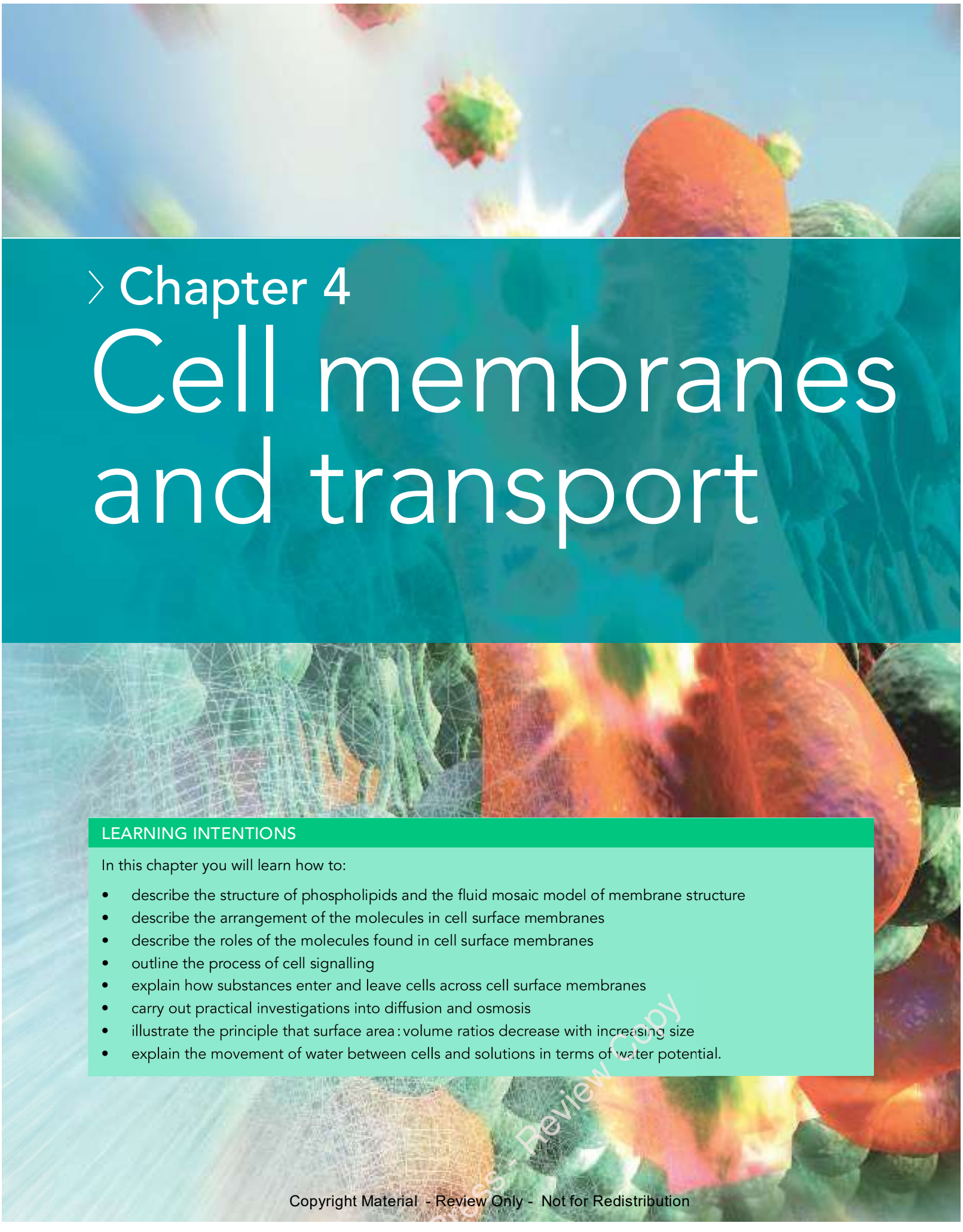
d Which inhibitor is competitive and which is non-competitive? Explain your answer. **[4]**

[Total: 11]

SELF-EVALUATION CHECKLIST

After studying this chapter, complete a table like this:

I can	See section...	Needs more work	Almost there	Ready to move on
state what enzymes are	3.1			
explain the mode of action of enzymes	3.2			
investigate the progress of enzyme-controlled reactions	3.3			
outline the use of a colorimeter for measuring the progress of enzyme-catalysed reactions	3.3			
investigate and explain the effect of temperature, pH, enzyme concentration and substrate concentration on the rate of enzyme-catalysed reactions	3.4			
use V_{max} and K_m to compare the affinity of different enzymes for their substrates	3.5			
explain the effects of reversible inhibitors, both competitive and non-competitive, on enzyme activity	3.6			
state the advantages of using immobilised enzymes	3.7			



Chapter 4
Cell membranes and transport

LEARNING INTENTIONS

In this chapter you will learn how to:

- describe the structure of phospholipids and the fluid mosaic model of membrane structure
- describe the arrangement of the molecules in cell surface membranes
- describe the roles of the molecules found in cell surface membranes
- outline the process of cell signalling
- explain how substances enter and leave cells across cell surface membranes
- carry out practical investigations into diffusion and osmosis
- illustrate the principle that surface area : volume ratios decrease with increasing size
- explain the movement of water between cells and solutions in terms of water potential.

BEFORE YOU START

- Why are *all* cells (prokaryotes and eukaryotes) surrounded by a cell surface membrane (Chapter 1)?
- What is the meaning of 'partially permeable' when applied to membranes (Chapter 1)?
- Why are phospholipids important in membrane structure (Chapter 2)?
- State **two** examples where the surface area of membranes has been increased by folding. For each example, state why the increased surface area is an advantage (Chapter 1).
- Give an example of a cell organelle that is surrounded by **a** a single membrane and **b** two membranes (an envelope) (Chapter 1).

DELIVERY BAGS

Liposomes are artificially prepared membrane-bound compartments (vesicles) (Figure 4.1). They can be prepared by breaking biological membranes into pieces, some of which re-seal themselves into balls resembling empty cells, though much smaller than cells on average.

Like intact cells, liposomes are surrounded by a phospholipid bilayer and the interior is usually aqueous. They were first described in 1961. Since then they have been used as artificial models of cells and, more importantly, for medical applications. In particular, they have been used to deliver drugs.

To do this, the liposome is made while in a solution of the drug, so the drug is inside the liposome. The liposome is then introduced into the body and, when it reaches a target cell, such as a cancer cell or other diseased cell, it fuses with that cell's surface membrane, delivering the drug inside the cell. Precise targeting can be achieved by inserting the correct recognition molecule – for example, an antigen or antibody – into the liposome membrane. Other targeting methods also exist.

In 2013 it was discovered that liposomes could provide a safe way to deliver the powerful anti-cancer drug staurosporine. Although this drug had been available since 1977, it had not been used because it kills any cells, including healthy ones, that it comes into contact with. This is because it interferes with several cell-signalling pathways.* The drug is added to liposomes and then disguising agents are added to the outer surfaces of the liposomes. These hide the drug from the immune system and allow it to target cancer cells only.

Liposomes have many other uses. For example, they are used in the cosmetics industry to deliver skin care products such as aloe vera, collagen, elastin and vitamins A and E when rubbed on skin. Liposome delivery of food supplements by mouth has also been tried with some success – absorption rates can be much higher than with traditional tablets.

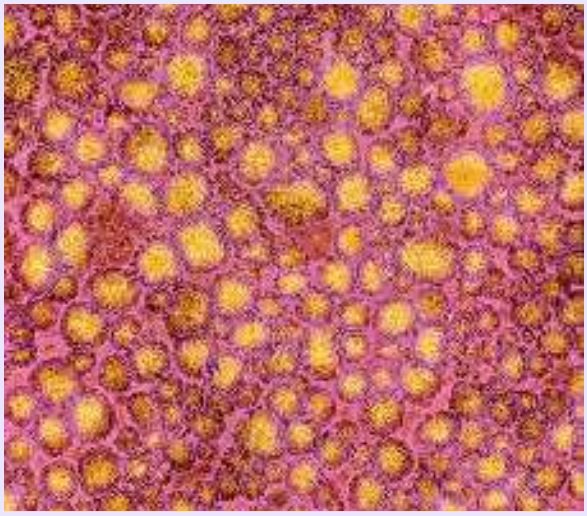


Figure 4.1: Liposomes.

Questions for discussion

Some of the trials with staurosporine have involved experiments on mice.

- What are the arguments for and against medical research on non-human animals?
- What rights do you think test animals should have?

* Staurosporine is a protein kinase inhibitor. The role of protein kinases in cell signalling is described in Chapter 14 (Section 14.4, The control of blood glucose).

4.1 The importance of membranes

In Chapter 1 you saw that all living cells are surrounded by a very thin membrane, the cell surface membrane. This controls the exchange of materials such as nutrients and waste products between the cell and its environment. Inside cells, regulation of transport across the membranes of organelles is also vital. Membranes have other important functions. For example, they enable cells to receive hormone messages and in chloroplasts they contain the light-absorbing pigments needed for photosynthesis. It is important to study the structure of membranes to understand how these functions are achieved.

4.2 Structure of membranes

Phospholipids

Understanding the structure of membranes depends on understanding the structure of phospholipids (see Chapters 1 and 2). Phospholipids help to form the membranes that surround cells and organelles.

Figure 4.2a shows what happens if phospholipid molecules are spread out over the surface of water. They form a single layer with their heads in the water, because the heads are polar (hydrophilic). Their tails project out of the water, because they are non-polar (hydrophobic). The term *polar* refers to an uneven distribution of charge. The significance of this is explained in Chapter 2 (Section 2.5, Lipids).

If the phospholipids are mixed with water, they form either:

- ball-like structures called micelles (Figure 4.2b), or
- sheet-like structures called bilayers (Figure 4.2c).

In a micelle, all the hydrophilic heads face outwards into the water. They shield the hydrophobic tails from the water. In the middle of the ball, the tails point in towards each other, creating a hydrophobic environment inside the micelle, as shown in Figure 4.2a. In bilayers, the hydrophobic tails are also shielded from the water by the hydrophilic heads, as shown in Figure 4.2c.

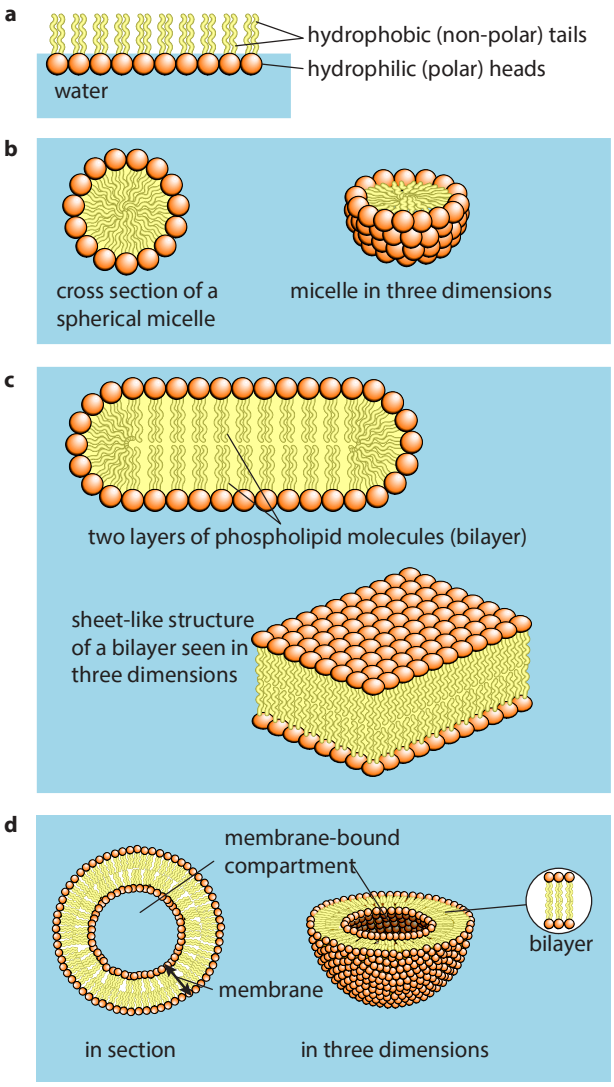


Figure 4.2: Phospholipids in water: **a** spread as a single layer of molecules (a monolayer) on the surface of water; **b** forming micelles surrounded by water; **c** forming bilayers; **d** bilayers forming membrane-bound compartments.

It is now known that the phospholipid bilayer is the basic structure of membranes, as shown in Figure 4.2d. 'Bilayer' means two layers, as shown in Figures 4.2c and d. The bilayer, or membrane, is about 7 nm wide (see Figure 1.22).

Membranes also contain proteins. The proteins can be seen in certain electron micrographs, such as Figure 4.3.

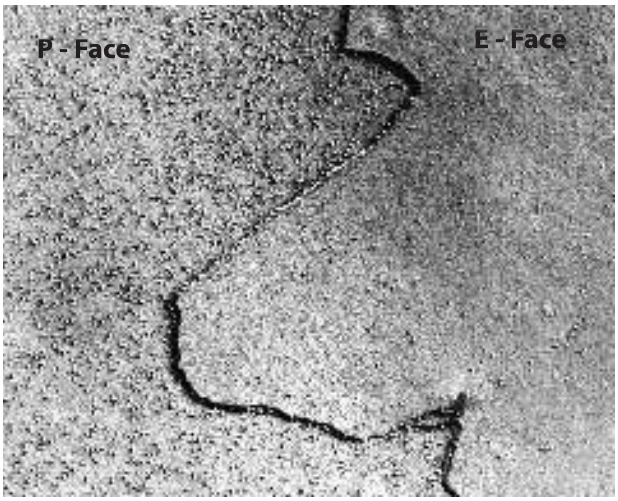


Figure 4.3: Scanning electron micrograph of a cell surface membrane. The membrane has been prepared by freeze-fracturing, which has split open the bilayer. The 'P-face' in the picture is the phospholipid layer nearest the inside of the cell and shows the many protein particles embedded in the membrane. The 'E-face' is part of the outer phospholipid layer (×50000).

The fluid mosaic model of membrane structure

In 1972 two scientists, Singer and Nicolson, used all the available evidence to put forward a hypothesis for membrane structure. They called their model the **fluid mosaic model**. It is described as 'fluid' because both the phospholipids and the proteins can move about by diffusion. The phospholipid bilayer has the sort of fluidity you associate with olive oil. The phospholipid molecules move sideways in their layers. Some of the protein molecules also move about within the phospholipid bilayer, like icebergs in the sea. Others remain fixed to structures inside or outside the cell. The word 'mosaic' describes the pattern produced by the scattered protein molecules when the surface of the membrane is viewed from above.

Figures 4.4 and 4.5 are diagrams of what scientists imagine a membrane might look like if you could see the individual molecules.

KEY WORD

fluid mosaic model: the currently accepted model of membrane structure, proposed by Singer and Nicolson in 1972, in which protein molecules are free to move about in a fluid bilayer of phospholipid molecules

Features of the fluid mosaic model

The membrane is a double layer (bilayer) of phospholipid molecules. The individual phospholipid molecules move about by diffusion within their layers.

The phospholipid tails point inwards, facing each other and forming a non-polar hydrophobic interior. The phospholipid heads face outwards into the aqueous (water-containing) medium that surrounds the membranes.

Some of the phospholipid tails are saturated and some are unsaturated. (Remember, unsaturated tails contain double bonds.) The more unsaturated they are, the more fluid the membrane. This is because the unsaturated fatty acid tails are bent (see Figure 2.11) and therefore fit together more loosely. Fluidity is also affected by tail length: the longer the tail, the less fluid the membrane. As temperature decreases, membranes become less fluid, but some organisms which cannot regulate their own temperature, such as bacteria and yeasts, respond by increasing the proportion of unsaturated fatty acids in their membranes.

Proteins may be found in the inner layer, the outer layer or, most commonly, spanning the whole membrane, in which case they are known as transmembrane proteins.

The proteins have hydrophobic (non-polar) and hydrophilic (polar) regions. They stay in the membrane because the hydrophobic regions, made from hydrophobic amino acids, are next to the hydrophobic fatty acid tails and are repelled by the

watery environment either side of the membrane. The hydrophilic regions, made from hydrophilic amino acids, are repelled by the hydrophobic interior of the membrane and therefore face into the aqueous environment inside or outside the cell, or line hydrophilic pores which pass through the membrane (see Figure 4.5).

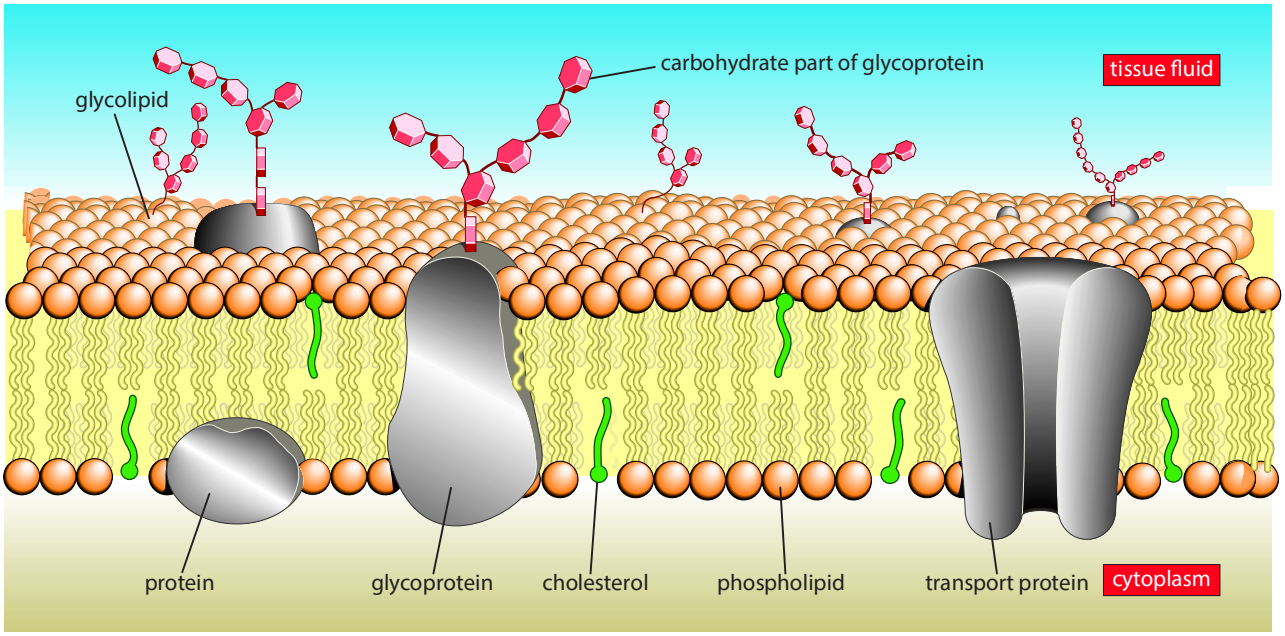


Figure 4.4: An artist's impression of the fluid mosaic model of membrane structure.

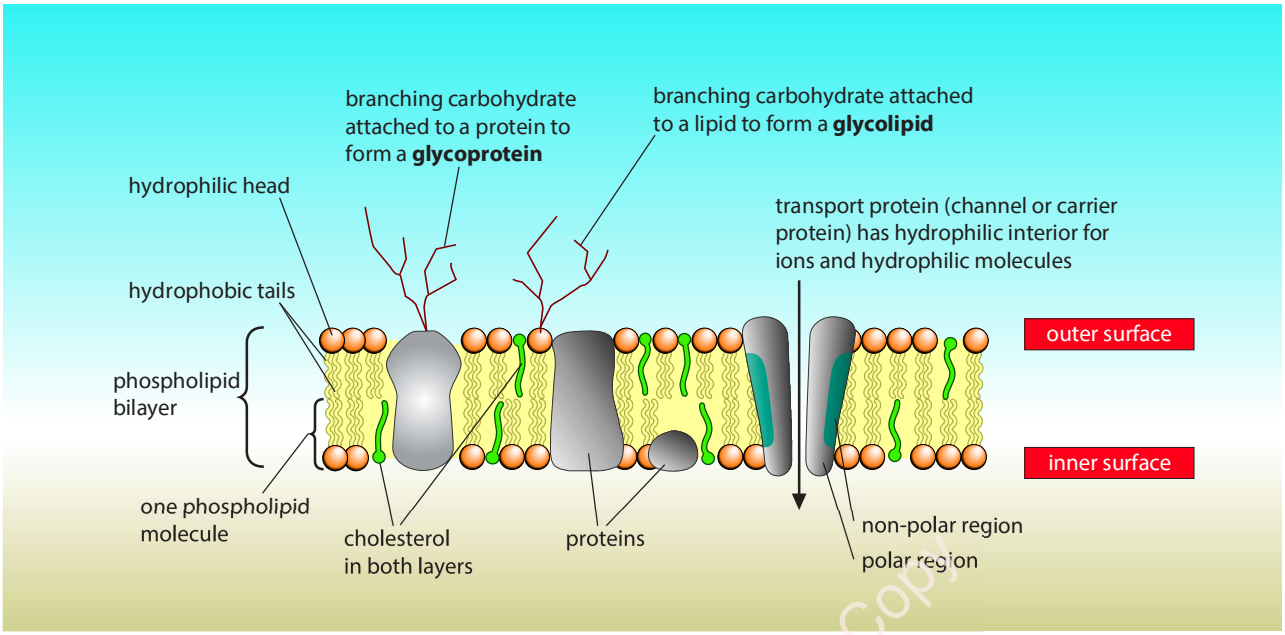


Figure 4.5: Diagram of the fluid mosaic model of membrane structure.

Most of the protein molecules float like icebergs in the phospholipid layers, although some are fixed to structures inside or outside the cell and do not move about.

Many proteins and lipids have short, branching carbohydrate chains, forming glycoproteins and glycolipids. The carbohydrate chains face outside the membrane.

Molecules of cholesterol are also found in the membrane.

4.3 Roles of the molecules found in membranes

You have seen that cell membranes contain several different types of molecule. There are three types of lipid: phospholipids, cholesterol and glycolipids. There are also proteins and glycoproteins. Each of these has a particular role to play in the overall structure and function of the membrane.

Phospholipids

As explained:

- phospholipids form a bilayer, which is the basic structure of the membrane
- fluidity of the membrane is affected by the length of the fatty acid tails and how saturated or unsaturated they are.

Because the tails of phospholipids are non-polar (hydrophobic), it is difficult for polar molecules or ions to pass through membranes. Membranes therefore act as a barrier to most water-soluble substances. This means that water-soluble molecules such as sugars, amino acids and proteins cannot leak out of the cell, and unwanted water-soluble molecules cannot enter the cell.

Some phospholipids can be modified to act as signalling molecules (see Section 4.4, Cell signalling).

Cholesterol

Cholesterol is a relatively small molecule. Like phospholipids, cholesterol molecules have hydrophilic heads and hydrophobic tails. They fit between the phospholipid molecules with their heads at the membrane surface. Cell surface membranes in animal cells contain almost as much cholesterol as phospholipid. Cholesterol is much less common in plant cell membranes and is absent from prokaryotes. In these organisms, compounds very similar to cholesterol serve the same function.

Cholesterol is important for the mechanical stability of membranes. It strengthens membranes by getting in between the phospholipid molecules and reducing fluidity. Without cholesterol, membranes quickly break and cells burst open.

The hydrophobic regions of cholesterol molecules help to prevent ions or polar molecules from passing through the membrane. This is particularly important in the myelin sheath, which surrounds nerve cells. The myelin sheath is made up of many layers of cell surface membrane. Leakage of ions would slow down nerve impulses.

At low temperatures the phospholipid tails tend to pack closer together, but cholesterol prevents this from happening too much. Maintaining the correct fluidity of the membrane means cells can survive colder temperatures.

Glycolipids, glycoproteins and proteins

Probably all the protein molecules and many of the lipid molecules on the outer surfaces of cell surface membranes have short carbohydrate chains attached to them. These 'combination' molecules are known as glycoproteins and glycolipids. Some of the functions of glycolipids and proteins, including glycoproteins, are summarised below.

Receptor molecules

The carbohydrate chains help the glycoproteins and glycolipids to act as receptor molecules. The function of receptor molecules is to bind with particular substances at the cell surface. Different cells have different receptors, depending on their function.

KEY WORD

cholesterol: a small, lipid-related molecule with a hydrophilic head and a hydrophobic tail which is an essential constituent of membranes; it is particularly common in animal cells and gives flexibility and stability to the membrane as well as reducing fluidity

One group of receptors are called 'signalling receptors', because they are part of a signalling system that coordinates the activities of cells. Signalling receptors recognise messenger molecules like hormones and neurotransmitters. (Neurotransmitters are discussed in Chapter 15, Section 15.2, Nervous communication.) When the messenger molecule binds to the signalling receptor, a series of chemical reactions is started inside the cell. An example of a signalling receptor is the glucagon receptor in liver cells (see Figure 14.26). Only cells that have glucagon receptors are affected by glucagon. Signalling is discussed further in Section 4.4, Cell signalling.

Cell-to-cell recognition

Some glycolipids and glycoproteins act as cell markers or antigens, allowing cells to recognise each other. The carbohydrate chains bind to complementary sites on other cells. Cell–cell recognition is important in growth and development and for immune responses. Each type of cell has its own type of antigen, rather like countries with different flags. For example, the ABO blood group antigens are glycolipids and glycoproteins which have small differences in their carbohydrate chains.

Transport proteins

Many proteins act as transport proteins. These provide hydrophilic channels or passageways for ions and polar molecules to pass through the membrane. Each transport protein is specific for a particular kind of ion or molecule. There are two types of transport protein: channel proteins and carrier proteins. Their roles are described in Section 4.5, Movement of substances across membranes.

Enzymes

Some membrane proteins are enzymes – for example, the digestive enzymes found in the cell surface membranes of the cells lining the small intestine. These catalyse the hydrolysis of molecules such as disaccharides.

Cytoskeleton

Some proteins on the inside of the cell surface membrane are attached to a system of protein filaments inside the cell known as the cytoskeleton. These proteins help to maintain and decide the shape of the cell. They may also be involved in changes of shape when cells move.

Other roles

Proteins also play important roles in the membranes of organelles. For example, in the membranes of mitochondria and chloroplasts they are involved in the processes of respiration and photosynthesis. (You will find out much more about this if you continue your biology course to A Level.)

Question

1 Prepare a table to summarise briefly the major functions of phospholipids, cholesterol, glycolipids, glycoproteins and proteins in cell surface membranes.

4.4 Cell signalling

Cell signalling is an important area of research in modern biology, with wide applications. It is important because it helps to explain how living organisms control and coordinate their bodies. In this chapter you will consider a few basic principles of signalling, especially the importance of membranes. Many features of cell signalling are shared between all living organisms.

KEY WORD

cell signalling: the molecular mechanisms by which cells detect and respond to external stimuli, including communication between cells

What is signalling? Basically, signalling is getting a message from one place to another.

Why do living organisms need signalling? All cells and organisms must be able to respond appropriately to their environments. This is made possible by means of signalling pathways which coordinate the activities of cells, even if they are large distances apart in the same body.

Signalling pathways can be electrical (e.g. the nervous system) or chemical (e.g. the hormone system in animals). They involve a wide range of molecules such as neurotransmitters and hormones. In this chapter you will concentrate on signalling pathways involving chemicals.

The first part of the pathway typically involves the following three main stages.

- A stimulus causes cells to secrete a specific chemical. The chemical is called a **ligand**. (The word ligand comes from the Latin word 'ligare', meaning to bind: you will see below that ligands bind to receptors.) The hormone glucagon is an example of a ligand. It is a specific chemical secreted by certain cells in the pancreas in response to a drop in blood sugar levels (the stimulus) (see Chapter 14, Section 14.4, The control of blood glucose).
- The ligand is transported to the target cells. Signalling molecules are usually relatively small for easy transport. In the case of hormones, the transport system is the blood system.
- The ligand binds to cell surface receptors on the target cells. The receptors are protein molecules located in the cell surface membrane.

The cell surface receptor is a specific shape and recognises the ligand. Only cells with this receptor can recognise the ligand. The ligand brings about a change in the shape of the receptor. The receptor spans the membrane, so the message is passed to the inside of the cell. Changing the shape of the receptor allows it to interact with the next component of the signalling pathway, so the message gets transmitted. Conversion of the original signal to a message that is then transmitted is called **transduction**.

KEY WORDS

ligand: a biological molecule which binds specifically to another molecule, such as a cell surface membrane receptor, during cell signalling

transduction: occurs during cell signalling and is the process of converting a signal from one method of transmission to another

The next component in the signalling pathway is often a 'G protein', which acts as a switch to bring about the release of a 'second messenger'. The second messenger is a small molecule which diffuses through the cell relaying the message. (G proteins are so-called because the switch mechanism involves binding to GTP (guanine triphosphate) molecules. GTP is similar to ATP, but with guanine in place of adenine.)

The stimulation of one receptor molecule results in many second messenger molecules being made in response. This represents an amplification (magnification) of the original signal, a key feature of signalling. The second messenger typically activates an enzyme, which in turn activates further enzymes, increasing the amplification at each stage. Finally, enzymes are produced which bring about the required change in cell metabolism.

The sequence of events triggered by the G protein is called a signalling cascade. Figure 4.6 is a diagram of a simplified cell-signalling pathway involving a second messenger. Examples of such a pathway involving the hormones glucagon and adrenaline are discussed in Chapter 14 (see Figure 14.26).

Apart from second messengers, there are three other basic ways in which a receptor can alter the activity of a cell:

- opening an ion channel, resulting in a change of membrane potential (e.g. nicotine-accepting acetylcholine receptors, Chapter 15)
- acting directly as a membrane-bound enzyme (e.g. glucagon receptor, Chapter 14)
- acting as an intracellular receptor when the initial signal passes straight through the cell surface membrane (e.g. the oestrogen receptor is in the nucleus and directly controls gene expression when combined with oestrogen).

Figure 4.7 summarises some typical signalling systems.

Some signalling molecules are hydrophobic. Examples include the steroid hormones (e.g. oestrogen). Figure 4.7 shows a hydrophobic signal molecule entering the cell. Hydrophobic signalling molecules can diffuse directly across the cell surface membrane and bind to receptors in the cytoplasm or nucleus.

Question

2 Why does the cell surface membrane not provide a barrier to the entry of hydrophobic molecules into the cell?

Direct cell-to-cell contact is another mechanism of signalling. This occurs, for example, when lymphocytes detect foreign antigens on other cells.

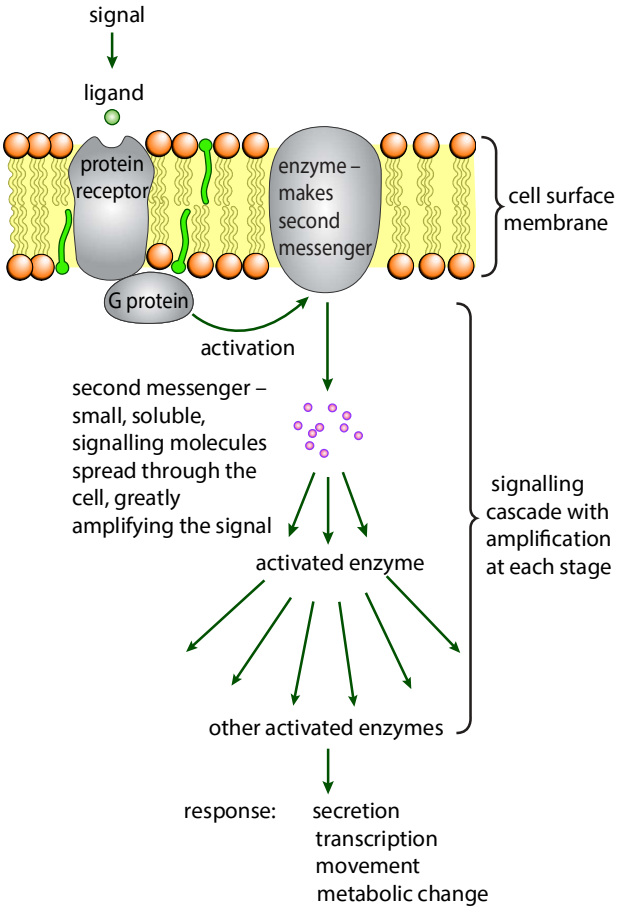


Figure 4.6: A simplified cell-signalling pathway involving a ligand and a second messenger.

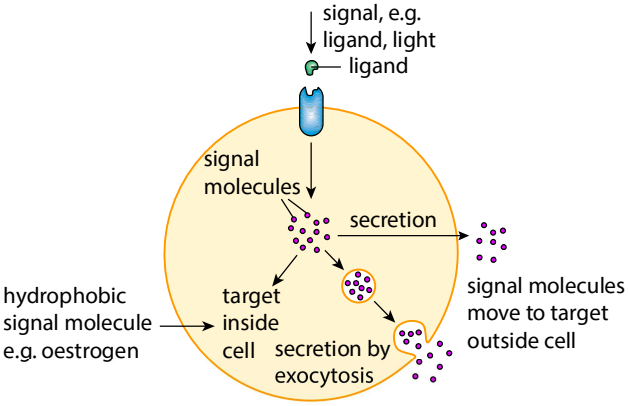


Figure 4.7: A few of the possible signalling pathways commonly found in cells.

4.5 Movement of substances across membranes

You have seen that a phospholipid bilayer around cells makes a very effective barrier, particularly against the movement of water-soluble molecules and ions. Such a barrier prevents the aqueous contents of the cell from escaping. However, some exchange between the cell and its environment is essential. There are five basic mechanisms by which this exchange is achieved:

- diffusion
- facilitated diffusion
- osmosis
- active transport
- bulk transport.

Question

3 Suggest **three** reasons why exchange between the cell and its environment is essential.

Diffusion

If you open a bottle of perfume in a room, it is not long before molecules of scent spread to all parts of the room (and are detected when they fit into membrane receptors in your nose). This will happen, even in still air, by the process of diffusion. **Diffusion** can be defined as the net movement of a substance from a region of its higher concentration to a region of its lower concentration as a result of random motion of its molecules or ions. The molecules or ions move down a concentration gradient. The random movement is caused by the natural kinetic energy (energy of movement) of the molecules or ions. As a result of diffusion, molecules or ions tend to reach an equilibrium situation where they are evenly spread

KEY WORD

diffusion: the net movement of molecules or ions from a region of higher concentration to a region of lower concentration down a concentration gradient, as a result of the random movements of particles

out within a given volume of space. Diffusion can be demonstrated easily using non-living materials such as glucose and Visking tubing (Practical Activity 4.1) or plant tissue (Practical Activity 4.2).

Some molecules or ions are able to pass through living cell membranes by diffusion. For example, the respiratory gases – oxygen and carbon dioxide – cross membranes by diffusion. They are uncharged and non-polar, and so can cross through the phospholipid bilayer between the phospholipid molecules. Water molecules, despite being very polar, can diffuse rapidly across the phospholipid bilayer because they are small enough.

PRACTICAL ACTIVITY 4.1

Demonstrating diffusion using Visking tubing

Visking tubing (also known as dialysis tubing) is a partially permeable, non-living membrane made from cellulose. It has molecular-sized pores which are small enough to prevent the passage of large molecules, such as starch and sucrose, but will allow the passage by diffusion of smaller molecules, such as glucose.

This can be demonstrated by filling a length of Visking tubing (about 15 cm) with a mixture of starch and glucose solutions. If the tubing is suspended in a test tube (or boiling tube) of water for a period of time, the presence of starch and glucose outside the tubing can be tested for at intervals to monitor whether diffusion out of the tubing has occurred. The results should indicate that glucose, but not starch, diffuses out of the tubing.

This experiment can be made more quantitative. It would be interesting, for example, to try to estimate the concentration of glucose outside the Visking tubing at different time intervals by setting up separate tubes, one for each planned time interval, and using a semi-quantitative Benedict's test each time. A colorimeter would be useful for this. Alternatively, a set of colour standards could be prepared. A graph could be drawn showing how the rate of diffusion changes with the concentration gradient between the inside and outside of the tubing.

You could design experiments in which sucrose and sucrase (an enzyme that breaks down sucrose) are added to the Visking tubing. You could also design experiments involving amylase, which breaks down starch.

(See Practical Investigation 4.2 in the Practical Workbook for additional information.)

PRACTICAL ACTIVITY 4.2

Demonstrating diffusion using plant tissue

Temporary staining of living plant cells – for example, adding iodine solution to epidermal cells to stain the nuclei and cytoplasm – shows that diffusion through cell surface membranes is possible. An experiment showing how the permeability of membranes is affected by environmental factors such as chemicals and temperature can be performed with beetroot.

Pieces of beetroot can be placed into water at different temperatures or into different alcohol concentrations. Any damage to the cell membranes results in the red pigment, which is normally contained within the large central vacuole, leaking out of the cells by diffusion. Changes in the colour of the surrounding solution can be monitored qualitatively or quantitatively. As in the experiment in Practical Activity 4.1, a colorimeter or a set of colour standards could be used. Alternatively, you could simply put the tubes in order and make up a colour scale (e.g. from 0 to 10), using water as 0 and the darkest solution as 10. There is an opportunity to design your own experiment.

What you are seeing is diffusion of the red dye from a region of high concentration in the vacuoles to a region of low concentration in the solution outside the pieces of beetroot. Diffusion is normally prevented by the partially permeable nature of the cell membranes.

(See Practical Investigation 4.8 in the Practical Workbook for additional information.)

Hydrophobic molecules can cross membranes because the interior of the membrane is hydrophobic.

The rate at which a substance diffuses across a membrane depends on a number of factors, including:

- steepness of the concentration gradient
- temperature
- the nature of the molecules or ions
- surface area.

The 'steepness' of the concentration gradient

The steeper the concentration gradient of a substance across a membrane, the faster the rate of diffusion of that substance. The steepness of the gradient is the difference in the concentration of the substance on the two sides of the membrane. If there are more molecules of the substance on one side of the membrane than on the other, there will be a net movement of molecules from where there are more to where there are fewer. Notice that it is a net movement. This means that although molecules move in both directions, more will move one way than the other depending on the gradient.

Temperature

At high temperatures, molecules and ions have much more kinetic energy than at low temperatures. They move faster, so diffusion is faster.

The nature of the molecules or ions

Large molecules require more energy to get them moving than small ones do, so large molecules tend to diffuse more slowly than small molecules. Non-polar molecules, such as glycerol, alcohol and steroid hormones, diffuse much more easily through cell membranes than polar ones, because they are soluble in the non-polar phospholipid tails.

The surface area across which diffusion is taking place

The greater the area of a surface, the more molecules or ions can cross it at any one moment, and therefore the faster diffusion can occur. The surface area of cell membranes can be increased by folding, as in microvilli in the cells lining the intestine or the cristae inside mitochondria.

An important factor is that the larger the cell, the smaller its surface area in relation to its volume. This is easily seen by studying Figure 4.8 in Question 4. To make the relevant calculations easier, cells are shown as cubes rather than spheres, but the principle remains the same: volume increases much more rapidly than surface area as size increases (see also Practical Activity 4.3). This has important implications for cells.

Question

4 Figure 4.8 shows three cubes.

Calculate the surface area, volume and surface area : volume ratio of each of the cubes.

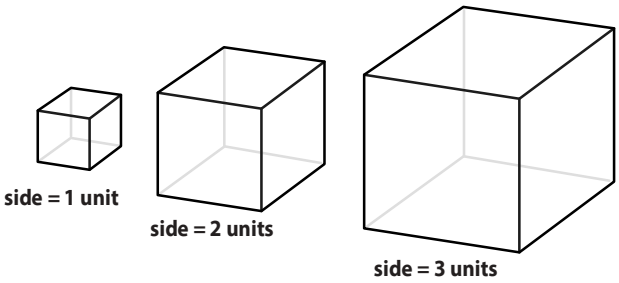


Figure 4.8: Diagram of three cubes.

The surface area : volume ratio decreases as the size of any three-dimensional (3D) object increases.

IMPORTANT

The surface area: volume ratio decreases as the size of any three-dimensional (3D) object increases.

Cells rely on diffusion because it is the main method by which molecules move about inside cells. This results in a limit on the size of cells, because the time taken to travel any distance by diffusion increases much faster than the distance does. Diffusion is, therefore, only effective over very short distances. An amino acid molecule, for example, can travel a few micrometres in several seconds, but would take several hours to diffuse 10 000 micrometres (a centimetre). Most eukaryotic cells are no larger than about 50 micrometres in diameter; prokaryotic cells are even smaller. An aerobic cell would quickly run out of oxygen and die if it were too large.

Question

5 The fact that surface area : volume ratio decreases with increasing size is also true for whole organisms. Explain the relevance of this for transport systems within organisms.

PRACTICAL ACTIVITY 4.3

Investigating the effect of size on diffusion

The effect of size on diffusion can be investigated by timing the diffusion of ions through blocks of agar of different sizes.

Solid agar is prepared in suitable containers such as ice cube trays. If the agar is made up with very dilute sodium hydroxide solution and Universal indicator, it will be coloured purple. Cubes of the required dimensions (e.g., sides of 2 cm × 2 cm, 1 cm × 1 cm, 0.5 cm × 0.5 cm) can be cut from the agar, placed in a container and covered with a diffusion solution such as dilute hydrochloric acid. (The acid should have a higher molarity than the sodium hydroxide so that its diffusion can be monitored by a change in colour of the indicator. Alternatively, the agar can be made up with Universal indicator only, although its colour will be affected by the pH of the water used.)

Either the time taken for the acid to completely change the colour of the indicator in the agar blocks, or the distance travelled into the block by the acid in a given time (e.g. 5 minutes), can be measured. The times can be converted to rates.

Finally, the rate of diffusion (rate of colour change) can be plotted against the surface area : volume ratio.

Using the same techniques, you may be able to design further experiments. For example, you could investigate the effect of the steepness of the concentration gradient on the rate of diffusion.

(See Practical Investigations 4.3 and 4.4 in the Practical Workbook for additional information.)

Facilitated diffusion

Large polar molecules, such as glucose and amino acids, cannot diffuse through the phospholipid bilayer. Nor can ions such as sodium (Na^+) or chloride (Cl^-). These can only cross the membrane with the help of certain protein molecules. Diffusion that needs help in this way is called **facilitated diffusion**. 'Facilitated' means made easy or made possible; the proteins facilitate the diffusion.

There are two types of transport protein involved, namely **channel proteins** and **carrier proteins**. Each channel protein or carrier protein is highly specific, allowing only one type of molecule or ion to pass through it.

Channel proteins

Channel proteins have water-filled pores as part of their structure. The pores allow charged substances, usually ions, to diffuse through the membrane. Most channel proteins are 'gated'. This means that part of the protein molecule on the inside surface of the membrane can move to close or open the pore, like a gate. This allows control of ion exchange. Two examples are the gated proteins found in nerve cell surface membranes. One type allows entry of sodium ions (Na^+), which happens during the production of an action potential (Chapter 15, Section 15.2,

KEY WORDS

facilitated diffusion: the diffusion of a substance through a transport protein (channel protein or carrier protein) in a cell membrane; the protein provides hydrophilic areas that allow the molecule or ion to pass through the membrane, which would otherwise be less permeable to it

channel protein: a membrane protein of fixed shape which has a water-filled pore through which selected hydrophilic ions or molecules can pass by facilitating diffusion or active transport

carrier protein: a membrane protein which changes shape to allow the passage into or out of the cell of specific ions or molecules by facilitated diffusion or active transport

Nervous communication). Another type allows exit of potassium ions (K^+) during the recovery phase, known as repolarisation. Some channels occur in a single protein; others are formed by several proteins combined. Some gated channel proteins require energy (in the form of ATP) to operate the gate.

Carrier proteins

Whereas channel proteins have a fixed shape, carrier proteins can flip between two shapes (Figure 4.9). As a result, the binding site is alternately open to one side of the membrane, then the other. This allows the molecule or ion to cross the membrane. Some carrier proteins change shape spontaneously. These are the ones that allow facilitated diffusion. Some carrier proteins, known as pumps, require energy and are involved in active transport (discussed later in this chapter).

Rate of diffusion through channel and carrier proteins

If molecules are diffusing across a membrane, the direction of movement depends on their relative concentration on each side of the membrane. They move down a concentration gradient from a higher to a lower concentration. However, the rate at which facilitated diffusion takes place is also affected by how many channel or carrier protein molecules there are in the membrane and, in the case of channel proteins, on whether they are open or not.

Osmosis

Osmosis is a special type of diffusion involving only water molecules. In the explanations that follow, remember that:

solute + solvent = solution

In a sugar solution, for example, the solute is sugar and the solvent is water.

KEY WORD

osmosis: the net diffusion of water molecules from a region of higher water potential to a region of lower water potential, through a partially permeable membrane

In Figure 4.10 there are two solutions separated by a partially permeable membrane. This is a membrane that allows only certain molecules through, just like membranes in living cells. In Figure 4.10a, solution **B** has a higher concentration of solute molecules than solution **A**. Solution **B** is described as more concentrated than solution **A**, and solution **A** is more dilute than solution **B**.

First, imagine what would happen if the membrane was not present. Both solute molecules and water molecules are free to move anywhere within the solutions. As they move randomly, both water molecules

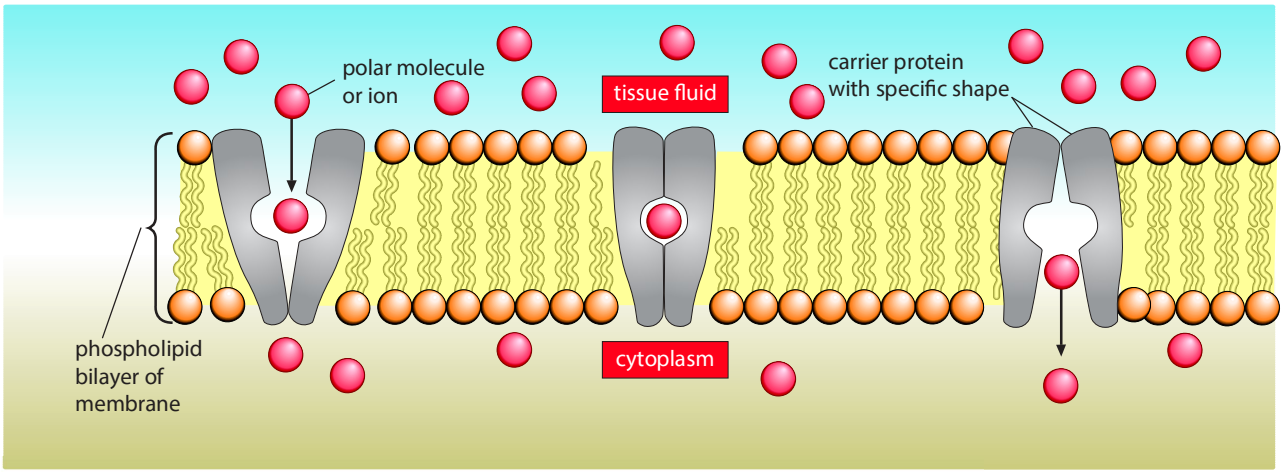


Figure 4.9: Changes in the shape of a carrier protein during facilitated diffusion. In this example, there is a net diffusion of molecules or ions into the cell down a concentration gradient.

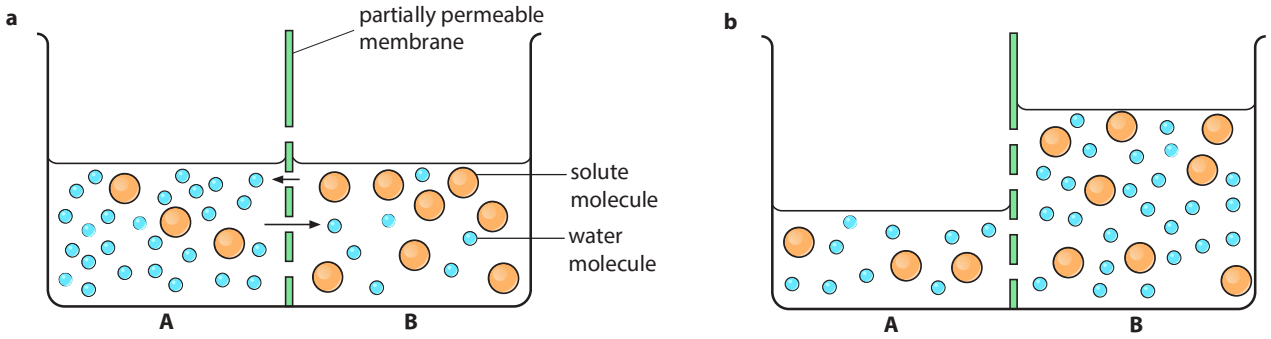


Figure 4.10: Two solutions separated by a partially permeable membrane. **a** Before osmosis. The solute molecules are too large to pass through the pores in the membrane, but the water molecules are small enough. **b** As the arrows in **a** show, more water molecules move from **A** to **B** than from **B** to **A**, so the net movement is from **A** to **B**, raising the level of solution in **B** and lowering it in **A**.

and solute molecules will tend to spread themselves evenly throughout the space available, by diffusion. At equilibrium, the concentration of the solution would be the same in **A** and **B**.

Now consider the situation where a partially permeable membrane *is* present, as in Figure 4.10. The solute molecules are too large to get through the membrane. Only water molecules can pass through. The solute molecules move about randomly, but as they hit the membrane they simply bounce back. The numbers of solute molecules each side of the membrane stay the same. The water molecules also move about randomly, but they are able to move both from **A** to **B** and from **B** to **A**. Over time, the water molecules will tend to spread themselves out more evenly between **A** and **B**.

This means that **A** will end up with fewer water molecules, so that the solution becomes more concentrated with solute. **B** will end up with more water molecules, so that it becomes more dilute. The volume of liquid in **B** will increase because it now contains the same number of solute molecules but has more water molecules. The solutions in **A** and **B** will have the same concentration.

The net diffusion of water molecules from a region of higher water potential to a region of lower water potential, through a partially permeable membrane, is called osmosis.

Water potential

The term **water potential** is very useful when considering osmosis. The Greek letter psi, ψ, can be used to mean water potential.

KEY WORD

water potential: a measure of the tendency of water to move from one place to another; water moves from a solution with higher water potential to one with lower water potential; water potential is decreased by the addition of solute, and increased by the application of pressure; the symbol for water potential is ψ or ψ_w

You can think of water potential as being the tendency of water to move from one place to another. Water *always* moves from a region of higher water potential to a region of lower water potential. So water always moves down a water potential gradient. For example, when water falls down a waterfall, it is moving from a higher water potential to a lower water potential.

Water will move until the water potential is the same throughout the system, at which point you can say that equilibrium has been reached.

With reference to osmosis, water potential depends on two factors:

- the concentration of the solution
- how much pressure is applied to it.

For example, a solution containing a lot of water (a dilute solution) has a higher water potential than a solution containing relatively little water (a concentrated solution). In Figure 4.10a, solution **A** has a higher water potential than solution **B**, because solution **A** is more dilute than solution **B**. This is why the net movement of water is from **A** to **B**. (high water potential to low water potential)

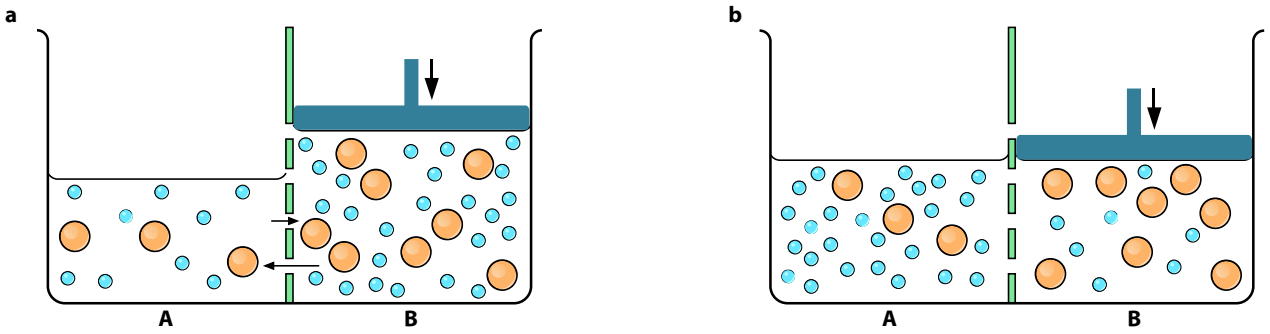


Figure 4.11: a Applying pressure to a solution increases the tendency of water to move out of it, so in this figure the pressure increases the water potential of the solution in **B**. **b** Water has moved from **B** to **A** as a result of the pressure. The equilibrium situation is shown. The pressure applied has returned the solutions to the situation shown in Figure 4.10a.

Now look again at Figure 4.10b. What would happen if you could now press down very hard on side **B**, as shown in Figure 4.11a?

Applying pressure to solution **B** makes it possible to 'squeeze' some of the water back into solution **A**. By increasing the pressure on solution **B**, you are increasing the tendency for water to move out of it – that is, you are increasing its water potential, until it is higher than the water potential in **A**. Pressure on a solution increases its water potential.

Measuring water potential

Water potential can be measured in pressure units called kiloPascals (abbreviated to kPa). As you have seen, the water potential of pure water will always be higher than the water potential of a solution (assuming there is no extra pressure applied to the solution). For reasons that you do not need to consider here, the water potential of pure water is set at 0 kPa. Since all solutions have a lower water potential than pure water, the water potential of all solutions must be less than zero. In other words their water potentials must be negative and the units will be –kPa. A dilute solution will have a less negative value than a concentrated solution. For example, a solution with a water potential of -10 kPa has a higher water potential than a solution with a water potential of -20 kPa.

Question

6 a In Figure 4.10b, the system has come into equilibrium, so there is no net movement of water molecules. What can you say about the water potentials of the two solutions **A** and **B**?

b i In Figure 4.11b, the solutions **A** and **B** are at equilibrium. Which solution, **A** or **B**, is more concentrated?

ii Why does water not move from the more dilute solution to the more concentrated solution?

Osmosis in animal cells

Figure 4.12 shows the effect of osmosis on an animal cell. A convenient type of animal cell to study in practical work is the red blood cell. A slide of fresh blood viewed with a microscope will show large numbers of red blood cells. Different samples of blood can be mixed with solutions of different water potential. Figure 4.12a shows that, if the water potential of the solution surrounding the cell is too high, the cell swells and bursts. If it is too low, the cell shrinks (Figure 4.12c). This shows one reason why it is important to maintain a constant water potential inside the bodies of animals.

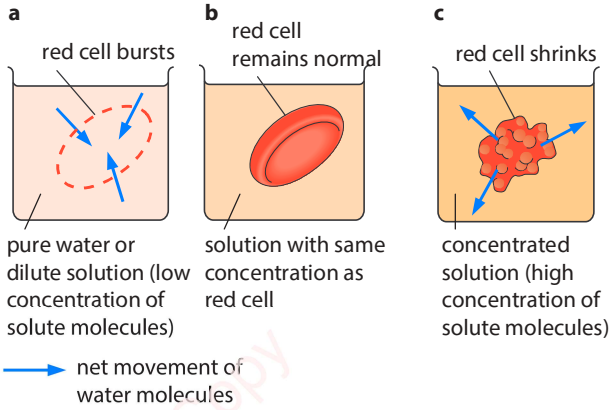


Figure 4.12: Movement of water into or out of red blood cells by osmosis in solutions of different concentration.

Question

7 In Figure 4.12:

a which solution has the highest water potential?

b in which solution is the water potential of the red cell the same as that of the solution?

Osmosis in plant cells

Unlike animal cells, plant cells are surrounded by cell walls, which are very strong and rigid. Imagine a plant cell being placed in pure water or a dilute solution (Figure 4.13a). The water or solution has a higher water potential than the plant cell, and water therefore enters the cell through its partially permeable cell surface membrane by osmosis. Just like in the animal cell, the volume of the cell increases, but in the plant cell the wall resists the expansion of the **protoplast**, which is the living part of the cell inside the cell wall surrounded by the cell surface membrane. Pressure rapidly starts to build up inside the cell. This pressure increases the water potential of the cell until the water potential inside the cell equals the water potential outside the cell, and equilibrium is reached (Figure 4.13b). The cell wall is so inelastic that the pressure builds up quickly and it takes very little water to enter the cell to achieve equilibrium. The cell wall prevents the cell from bursting, unlike the situation when an animal cell is placed in pure water or a dilute solution. When a plant cell is fully inflated with water, it is described as turgid.

Figure 4.13c shows the situation when a plant cell is placed in a solution of lower water potential. An example would be a concentrated sucrose solution. In such a solution, water will leave the cell by osmosis. As it does so, the protoplast gradually shrinks until it is exerting no pressure at all on the cell wall. Both the solute molecules and the water molecules of the external solution can continue to pass through the freely permeable cell wall, and so the external solution remains in contact with the shrinking protoplast.

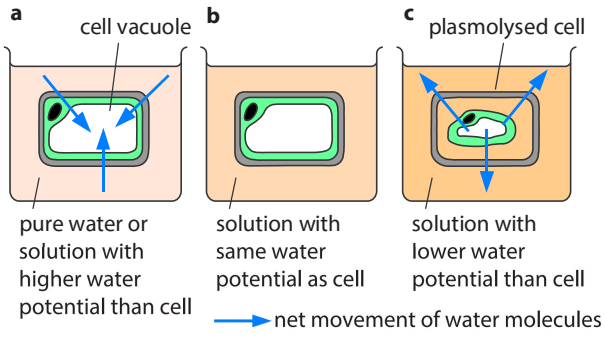


Figure 4.13: Osmotic changes in a plant cell in solutions of different water potential.

As the protoplast continues to shrink, it begins to pull away from the cell wall (Figures 4.14 and 4.15). This process is called **plasmolysis**, and a cell in which it has happened is said to be plasmolysed (Figures 4.13c, 4.14 and 4.15). The point at which plasmolysis is about to occur is referred to as **incipient plasmolysis**. This is the point when the protoplast no longer exerts any pressure on the cell wall. Eventually, as with the animal cell, an equilibrium is reached when the water potential of the cell has decreased until it equals that of the external solution.

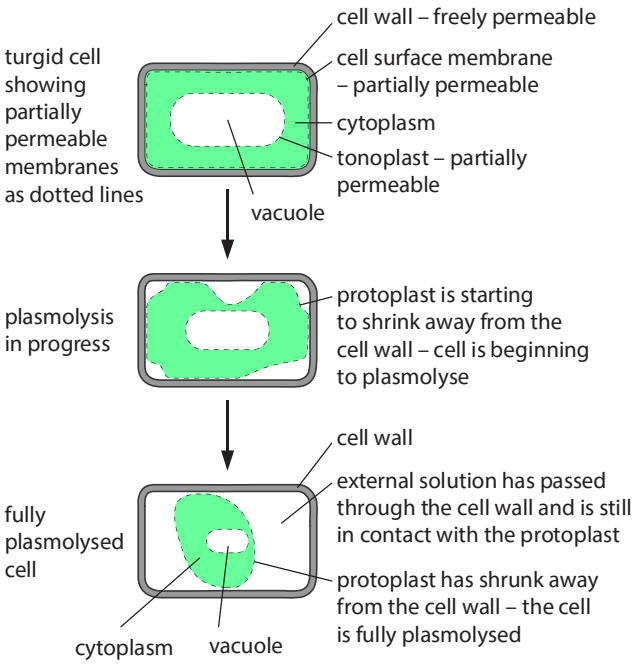


Figure 4.14: How plasmolysis occurs.

KEY WORDS

protoplast: the living contents of a plant cell, including the cell surface membrane but excluding the cell wall

plasmolysis: the loss of water from a plant or prokaryote cell to the point where the protoplast shrinks away from the cell wall

incipient plasmolysis: the point at which plasmolysis is about to occur when a plant cell or a prokaryote cell is losing water; at this point the protoplast is exerting no pressure on the cell wall

The changes described can easily be observed with a light microscope using strips of epidermis peeled from rhubarb petioles or from the swollen storage leaves of onion bulbs (Figure 4.15). The epidermal strips could be placed in a range of sucrose solutions of different concentration to find out which cause plasmolysis (Practical Activity 4.4).

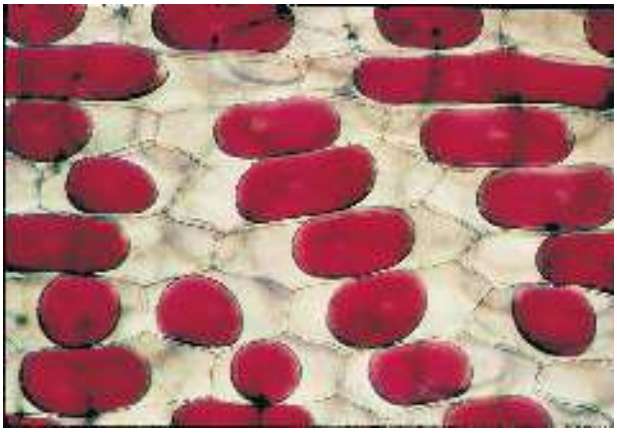


Figure 4.15: Light micrograph of red onion cells that have plasmolysed (×100).

Questions

8 Figures 4.14 and 4.15 show a phenomenon called plasmolysis. Why can plasmolysis not take place in an animal cell?

9 Inflating a balloon can mimic water entering a cell by osmosis.

- **a** What does the skin of the balloon represent?
- **b** What will happen if inflation continues (animal cell)?
- **c** What will happen if the balloon is inside a strong box (plant cell)?

10 Two neighbouring plant cells are shown in Figure 4.16. Cell A has a higher water potential than cell B. (Remember, the closer the water potential is to zero, the higher it is.)

- **a** In which direction would there be net movement of water molecules?
- **b** Explain what is meant by 'net movement'.
- **c** Explain your answer to **a**.
- **d** Explain what would happen if both cells were placed in:
  - **i** pure water
  - **ii** a sucrose solution with a lower water potential than either cell.

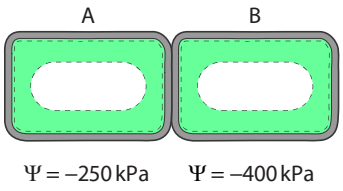


Figure 4.16 Water potential in two neighbouring plant cells.

PRACTICAL ACTIVITY 4.4

Investigating osmosis in plant cells

Observing osmosis in plant cells

Epidermal strips are useful material for observing plasmolysis. Coloured sap makes observation easier. Suitable sources are the inner surfaces of the fleshy storage leaves of red onion bulbs, rhubarb petioles and red cabbage leaves.

The strips of epidermis may be placed in a range of molarities of sucrose solution (up to 1.0 mol dm^{-3}) or sodium chloride solutions of up to 3%. Small pieces of the strips can then be placed on glass slides, mounted in the relevant solution, and observed with a microscope. Plasmolysis may take several minutes, if it occurs.

Determining the water potential of a plant tissue

The principle in this experiment is to find a solution of known water potential which will cause neither a gain nor a loss in water of the plant tissue being examined. Samples of the tissue – for example, potato chips of standard size – are allowed to come into equilibrium with a range of solutions of different water potentials. Sucrose solutions may be used. Changes in either mass or volume are recorded. Length of chips can be used as a measure of volume if chips are all the same dimensions. Plotting a graph of the results allows the solution that causes no change in mass or volume to be determined. This solution will have the same water potential as the plant tissue.

(See Practical Investigations 4.5, 4.6 and 4.7 in the Practical Workbook for additional information.)

11 In an experiment to determine the water potential of fresh beetroot tissue, a student cut 12 rectangular 'chips' of tissue approximately 2 mm thick, 5 mm wide and 50 mm long, taken from the middle of a large beetroot. Two chips were immersed in each of six covered Petri dishes, one containing water and the others containing solutions of sucrose of different molarity, up to a maximum of 1 mol dm^{-3}. The lengths of the chips were then measured accurately against graph paper seen through the bottoms of the dishes. Mean percentage changes in length of the chips were then measured after six hours.

- **a** Why was it important to use fresh beetroot tissue in this experiment?
- **b** Why would it have been important to immerse the beetroot chips as soon as possible after they were cut?
- **c** Suggest why length was measured rather than volume.
- **d** Why were at least two chips added to each dish?
- **e** Why were the dishes covered when left?
- **f** Suggest **one** advantage of measuring change in length rather than change in mass of the chips in this experiment.
- **g** Suggest **one** advantage of measuring change in mass rather than change in length.

Active transport

Certain ions, such as potassium and chloride ions, are often found to be 10–20 times more concentrated inside cells than outside. In other words, a concentration gradient exists, with a lower concentration outside and a higher concentration inside the cell. The ions inside the cell originally came from the external solution. Therefore diffusion cannot be responsible for the gradient because, as you have seen, ions diffuse from high concentration to low concentration. The ions must therefore build up *against* a concentration gradient.

The process responsible is called **active transport**. It is achieved by carrier proteins called pumps, each of which is specific for a particular type of molecule or ion. However, unlike facilitated diffusion, active transport requires energy because movement occurs up a concentration gradient rather than down. The energy is most often supplied by the molecule ATP (adenosine triphosphate) which is produced during respiration inside the cell. The energy is used to make the carrier protein change its shape, transferring the molecules or ions across the membrane in the process (Figure 4.17).

An example of a carrier protein used for active transport is the **sodium–potassium pump** (**Na^+–K^+ pump**) (Figure 4.18). Such pumps are found in the cell surface membranes of all animal cells. In most cells, they run

KEY WORDS

active transport: the movement of molecules or ions through transport proteins across a cell membrane, against their concentration gradient, using energy from ATP

sodium–potassium pump (Na⁺–K⁺ pump): a membrane protein (or proteins) that moves sodium ions out of a cell and potassium ions into it, using ATP

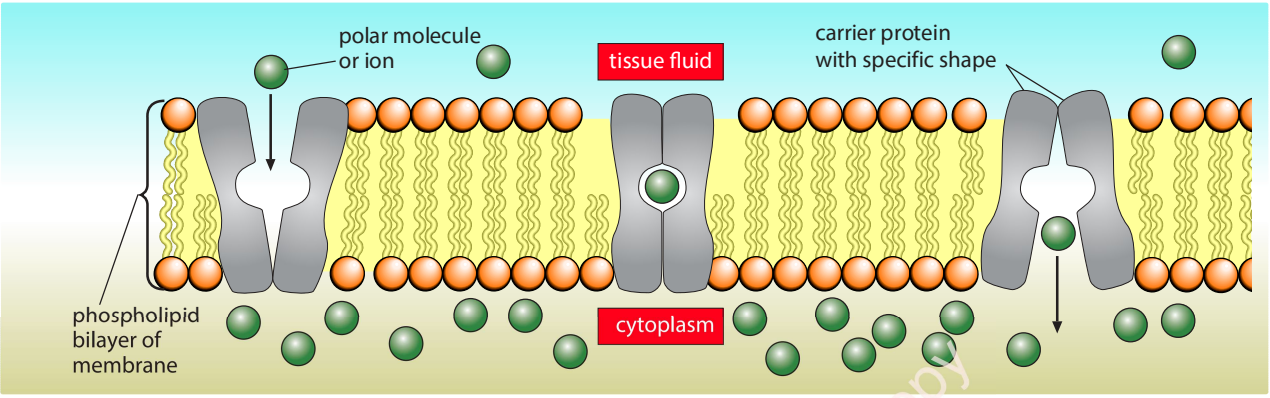


Figure 4.17: Changes in the shape of a carrier protein during active transport. Here, molecules or ions are being pumped into the cell *against* a concentration gradient. (Compare Figure 4.9.)

all the time, and it is estimated that on average they use 30% of a cell's energy (70% in nerve cells).

The role of the sodium–potassium pump is to pump three sodium ions out of the cell at the same time as allowing two potassium ions into the cell for each ATP molecule used. Sodium and potassium ions are both positively charged, so the net result is that the inside of the cell becomes more negative than the outside. A potential difference (p.d.) is created across the membrane. The significance of this in nerve cells is discussed in Chapter 15 (Section 15.2, Nervous communication).

In Figure 4.18 you can see that the pump has a receptor site for ATP on its inner surface (coloured red in the diagram). The receptor site acts as an ATPase enzyme in bringing about the hydrolysis of ATP to ADP (adenosine diphosphate) and phosphate to release energy.

Active transport can therefore be defined as the energy-consuming transport of molecules or ions across a membrane against a concentration gradient (from a lower to a higher concentration). The energy is provided by ATP from cell respiration. Active transport can occur either into or out of the cell.

Active transport is important in reabsorption in the kidneys, where certain useful molecules and ions have to be reabsorbed into the blood after filtration into the kidney tubules. It is also involved in the absorption of some products of digestion from the gut. In plants, active transport is used to load sugar from the photosynthesising cells of leaves into the phloem tissue for transport around the plant (Chapter 7, Section 7.5, Transport of assimilates), and to load inorganic ions from the soil into root hairs.

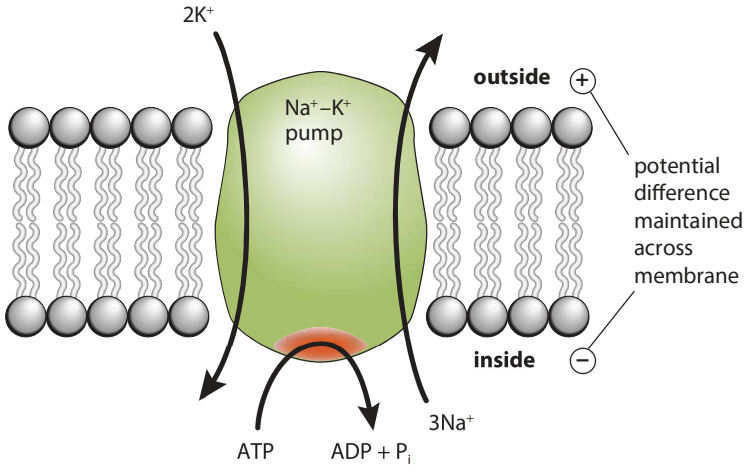


Figure 4.18: The sodium–potassium pump (Na^+–K^+ pump).

Endocytosis and exocytosis

So far you have been looking at ways in which individual molecules or ions cross membranes. Sometimes cells need to transport materials across their cell surface membranes on a much larger scale than is possible using the mechanisms studied so far. The materials include large molecules such as proteins or polysaccharides, parts of cells or even whole cells. As a result, mechanisms have evolved for the bulk transport of large quantities of materials into and out of cells.

Bulk transport of materials into cells is called **endocytosis**. Bulk transport out of cells is called **exocytosis**. These processes require energy.

Endocytosis

In endocytosis the cell surface membrane engulfs material to form a small sac (also known as a vesicle or a vacuole). Endocytosis takes two forms.

KEY WORDS

endocytosis: the bulk movement of liquids (pinocytosis) or solids (phagocytosis) into a cell, by the infolding of the cell surface membrane to form vesicles containing the substance; endocytosis is an active process requiring ATP

exocytosis: the bulk movement of liquids or solids out of a cell, by the fusion of vesicles containing the substance with the cell surface membrane; exocytosis is an active process requiring ATP

- Phagocytosis or 'cell eating' – this is the bulk uptake of solid material. Cells specialising in this are called **phagocytes**. The process is called phagocytosis and the vacuoles are called phagocytic vacuoles. An example is the engulfing of bacteria by white blood cells (Figure 4.19).
- Pinocytosis or 'cell drinking' – this is the bulk uptake of liquid. The vacuoles or vesicles formed are often extremely small, in which case the process is called micropinocytosis.

KEY WORD

phagocyte: a type of cell that ingests (eats) and destroys pathogens or damaged body cells by the process of phagocytosis; some phagocytes are white blood cells

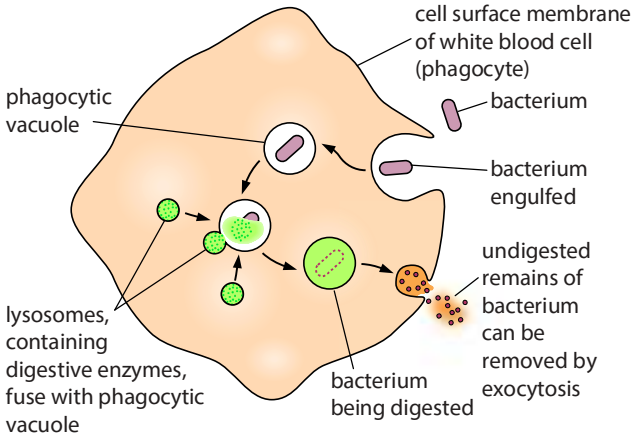


Figure 4.19: Stages in phagocytosis of a bacterium by a white blood cell.

Exocytosis

Exocytosis is the reverse of endocytosis and is the process by which materials are removed from cells (Figure 4.20). It happens, for example, in the secretion of digestive enzymes from cells of the pancreas (Figure 4.21). Secretory vesicles from the Golgi apparatus carry the enzymes to the cell surface and release their contents. Plant cells use exocytosis to get their cell wall building materials to the outside of the cell surface membrane.

Question

12 There are more mitochondria than average in pancreatic acinar cells. Suggest a reason for this. (See Figure 4.21 and also Exam-style Question 9 in Chapter 1.)

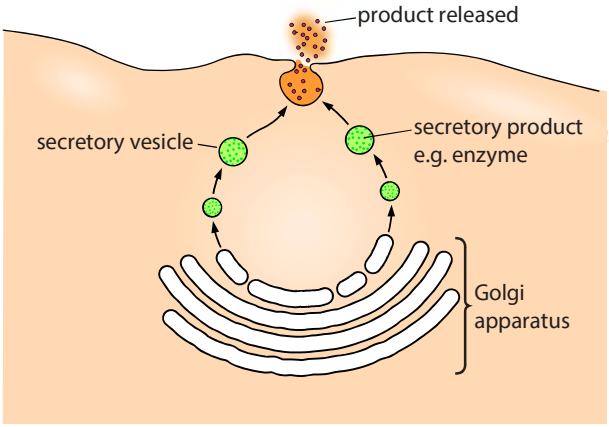


Figure 4.20: Exocytosis in a secretory cell. If the product being secreted is a protein, the Golgi apparatus is often involved in chemically modifying the protein before it is secreted, as in the secretion of digestive enzymes by the pancreas.

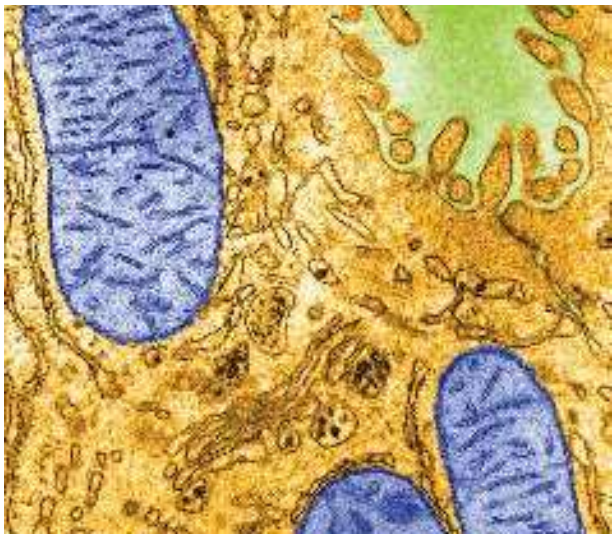


Figure 4.21: Transmission electron micrograph (TEM) of a pancreatic acinar cell secreting protein. The outside of the cell is coloured green. Golgi vesicles (secretory vesicles) with darkly stained contents can be seen making their way from the Golgi apparatus to the cell surface membrane. Mitochondria are coloured blue.

REFLECTION

The following exercise will help you to consider some of the connections that can be made between the many new biological terms you have studied in the first four chapters of this book.

For each pair of terms below, find a suitable word or term that links the pair. Also try to explain the connection between the pairs. For example:

mitochondrion – ATP: The missing term could be 'cell respiration' because cell respiration takes place in mitochondria and the product of cell respiration is ATP.

β-glucose – cell wall

monomer – protein

Michaelis–Menten constant – affinity

centrosome – microtubule

animal storage product – 1,4 and 1,6 linkages

nm – mm

Golgi apparatus – hydrolytic enzymes

ribosome – amino acid

amino acid – protein

α-glucose – plant storage product

monomer – polymer

microtubule – 9 + 2

induced fit – lock and key

peptidoglycan – cellulose

pinocytosis – endocytic vacuole

fatty acid – triglyceride

ligand – G protein

nucleolus – protein synthesis

The following grid shows some more terms that can be linked in some way. For example:

- hydrogen bond can be linked in some way with water and also with protein.
- fluid mosaic model can be linked with four terms.

A short line between two terms shows that they can be linked. Try to explain links.

glycolipid		phospholipid		hydrophilic
water		fluid mosaic model		facilitated diffusion
hydrogen bond		protein		active transport

Personal reflection question

In what ways do you think you need to improve based on your performance in this activity?

Final reflection

Discuss with a friend which, if any, parts of Chapter 4 you need to:

- read through again to make sure you really understand
- seek more guidance on, even after going over it again.

SUMMARY

The basic structure of a membrane is a 7 nm thick phospholipid bilayer containing protein molecules. The structure is described as a fluid mosaic.
Phospholipid bilayers are a barrier to most water-soluble substances because the interior of the membrane is hydrophobic.
Cholesterol is also needed for membrane fluidity and stability.
Some proteins are transport proteins, transporting molecules or ions across the membrane. They may be either channel proteins or carrier proteins. Channel proteins have a fixed shape; carrier proteins change shape. Some proteins act as enzymes.
Glycolipids and glycoproteins form receptors – for example, for hormones or neurotransmitters. Glycolipids and glycoproteins also form cell–cell recognition markers.
Membranes play an important role in cell signalling, the means by which cells communicate with each other.
The cell surface membrane controls exchange between the cell and its environment. Some chemical reactions take place on membranes inside cell organelles, as in photosynthesis and respiration.
Diffusion is the net movement of molecules or ions from a region of their higher concentration to one of lower concentration. Oxygen, carbon dioxide and water cross membranes by diffusion. Diffusion of ions and polar molecules through membranes is allowed by transport proteins. This process is called facilitated diffusion.
Water moves from regions of higher water potential to regions of lower water potential. When water moves from regions of higher water potential to regions of lower water potential through a partially permeable membrane, such as the cell surface membrane, this diffusion is called osmosis.
Adding solute lowers the water potential. Adding pressure to a solution increases the water potential.
In dilute solutions, animal cells burst as water moves into the cytoplasm from the solution. In dilute solutions, a plant cell does not burst, because the cell wall provides resistance to prevent it expanding. In concentrated solutions, animal cells shrink, while in plant cells the protoplast shrinks away from the cell wall in a process known as plasmolysis.
Some ions and molecules move across membranes by active transport, against the concentration gradient. This needs a carrier protein and ATP to provide energy. Exocytosis and endocytosis involve the formation of vacuoles to move larger quantities of materials respectively out of, or into, cells by bulk transport. There are two types of endocytosis, namely phagocytosis (cell eating) and pinocytosis (cell drinking).

EXAM-STYLE QUESTIONS

1 What are the most abundant molecules in the cell surface membranes of plant cells?

A cholesterol
B glycolipids
C phospholipids
D proteins [1]

CONTINUED

2 Where are the carbohydrate portions of glycolipids and glycoproteins located in cell surface membranes?

A the inside and outside surfaces of the membrane
B the inside surface of the membrane
C the interior of the membrane
D the outside surface of the membrane [1]

3 In a signalling pathway, which of the following types of protein acts as a switch to release a second messenger?

A enzyme
B glycoprotein
C G protein
D receptor [1]

4 One role of cholesterol in membranes is:

A cell recognition
B cell-signalling receptor
C controlling fluidity
D hydrophilic channel [1]

5 **a** Describe fully what will occur if a plant cell is placed in a solution that has a higher water potential than the cell. Use the following terms in your answer:

cell wall, freely permeable, partially permeable, cell surface membrane, vacuole, tonoplast, cytoplasm, water potential, turgid, osmosis, protoplast, equilibrium

[12]

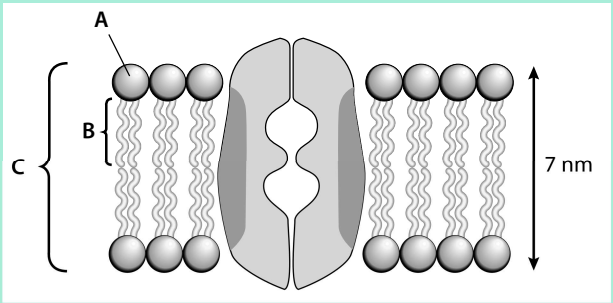
b Describe fully what will occur if a plant cell is placed in a solution that has a lower water potential than the cell. Use the following terms in your answer:

cell wall, freely permeable, partially permeable, cell surface membrane, vacuole, tonoplast, cytoplasm, water potential, incipient plasmolysis, plasmolysed, osmosis, protoplast, equilibrium

[13]

[Total: 25]

6 The diagram below shows part of a membrane containing a channel protein.



a Identify the parts labelled A, B and C. [3]

CONTINUED

b For each of the following, state whether the component is hydrophilic or hydrophobic:

i A

ii B

iii darkly shaded part of protein

iv lightly shaded part of protein **[2]**

c Explain how ions would move through the channel protein. **[3]**

d State **two** features that the channel proteins and carrier proteins of membranes have in common, apart from being proteins. **[2]**

e State **one** structural difference between channel and carrier proteins. **[1]**

f Calculate the magnification of the drawing. Show your working. **[4]**

[Total: 15]

7 Copy the table below and place a tick or cross in each box as appropriate.

Process	Uses energy in the form of ATP	Uses proteins	Specific	Controllable by cell
diffusion				
osmosis				
facilitated diffusion				
active transport				
endocytosis and exocytosis				

[20]

8 Copy and complete the table below to **compare** cell walls with cell membranes.

Feature	Cell wall	Cell membrane
is the thickness normally measured in nm or μm?		
cell location		
permeability		
fluid or rigid		

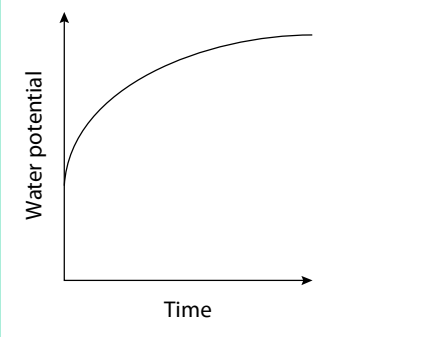
[4]

COMMAND WORD

Compare: identify / comment on similarities and/or differences.

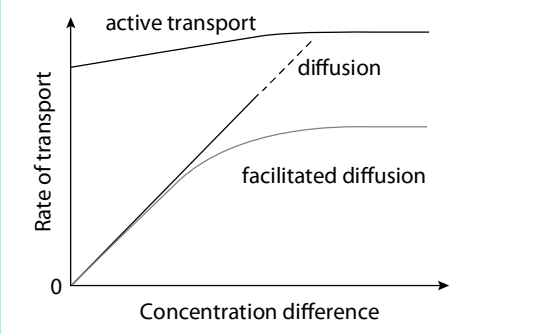
CONTINUED

9 A plant tissue was placed in pure water at time zero. The rate of entry of water into the tissue was measured as the change in water potential with time. The graph shows the results of this investigation.



Describe and explain the results obtained. **[8]**

10 The rate of movement of molecules or ions across a cell surface membrane is affected by the relative concentrations of the molecules or ions on either side of the membrane. The graph below shows the effect of concentration difference (the steepness of the concentration gradient) on three transport processes: diffusion, facilitated diffusion and active transport.



a **With reference to the graphs**, state what the **three** transport processes have in common. **[1]**

b Describe the rates of transport observed when the concentration difference is zero. **[2]**

c Explain the rates of transport observed when the concentration difference is zero. **[2]**

d **i** Which **one** of the processes would stop if a respiratory inhibitor was added? **[1]**

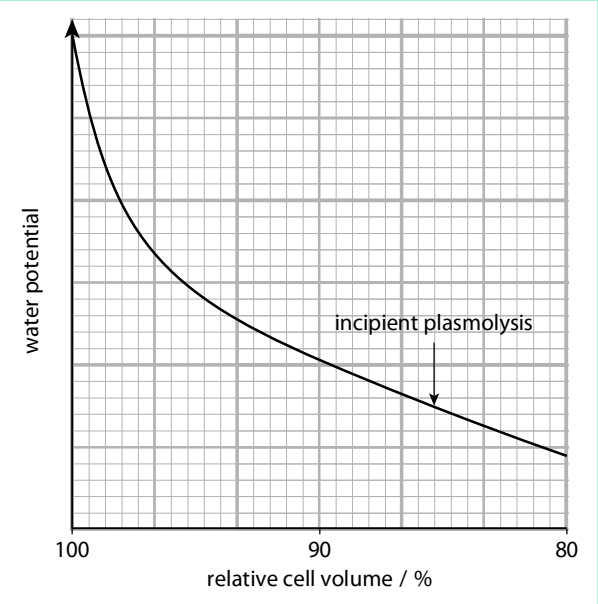
ii Explain your answer. **[2]**

e Suggest an explanation for the difference between the graphs for diffusion and facilitated diffusion. **[5]**

[Total: 13]

CONTINUED

11 When a cell gains or loses water, its volume changes. The graph shows changes in the water potential (ψ) of a plant cell as its volume changes as a result of gaining or losing water. (Note that 80% relative cell volume means the cell or protoplast has shrunk to 80% of the volume it was at 100% relative cell volume.)



a What is a protoplast? **[1]**

b **i** State the relative cell volume when the cell is at maximum turgidity. **[1]**

ii Describe what happens inside the cell as relative cell volume increases. **[5]**

c The graph shows the point of incipient plasmolysis.

i State the relative cell volume at incipient plasmolysis. **[1]**

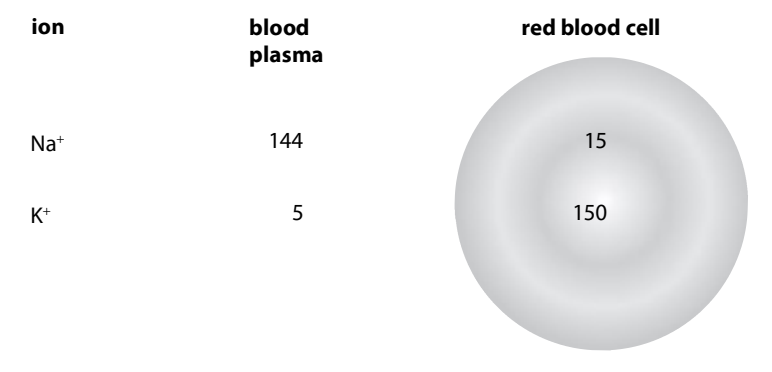
ii State what is meant by the term *incipient plasmolysis*. **[1]**

iii Describe what is happening to the cell between the point of incipient plasmolysis and the point where it has shrunk to a relative volume of 80%. **[3]**

[Total: 12]

CONTINUED

12 The diagram shows the concentration in mmol dm^{-3} of two different ions inside a human red blood cell and in the plasma outside the cell.



a Explain why these concentrations could not have occurred as a result of diffusion. **[1]**

b Explain how these concentrations could have been achieved. **[2]**

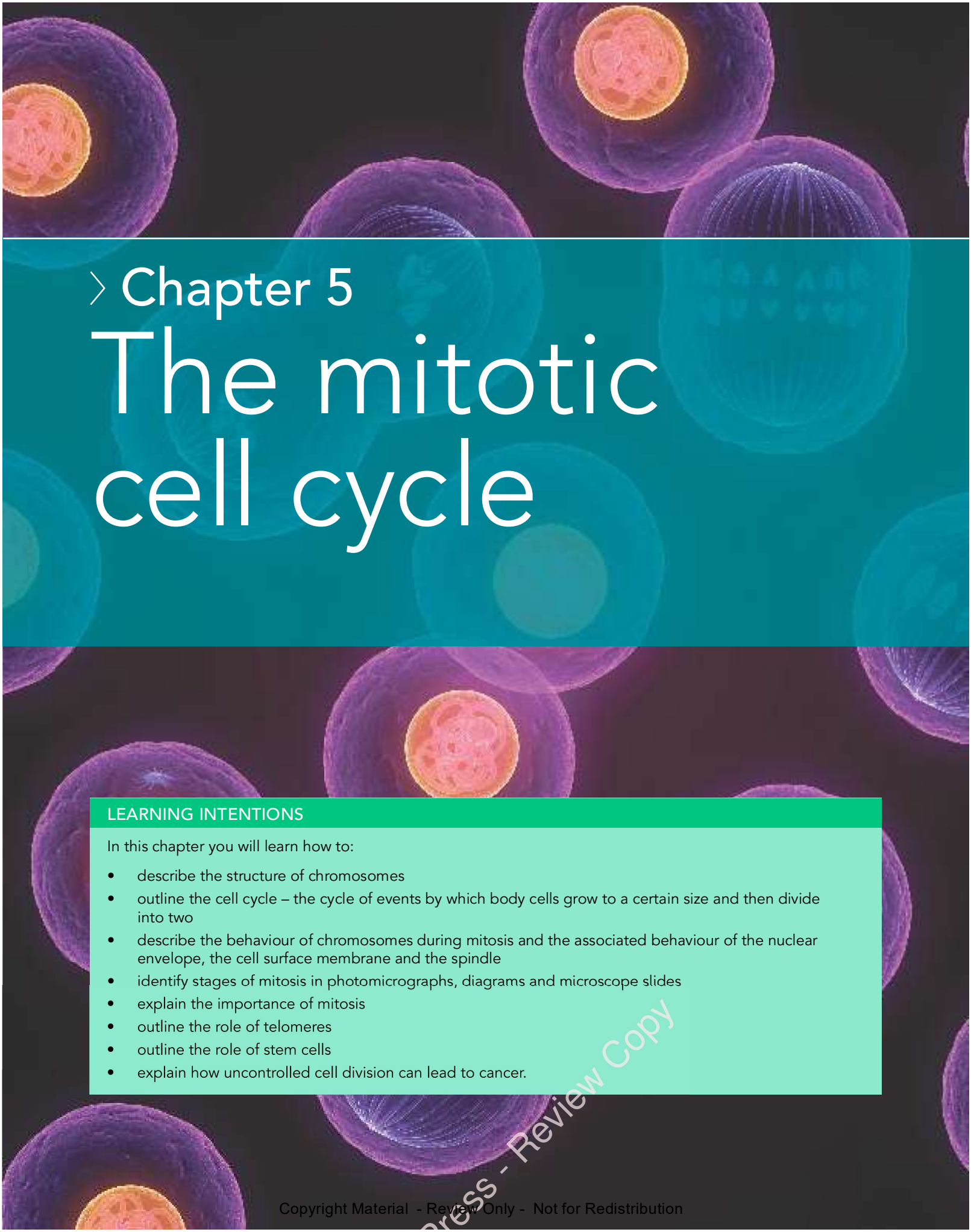
c If respiration of red blood cells is inhibited, the concentrations of potassium ions and sodium ions inside the cells gradually change until they come into equilibrium with the plasma. Explain this observation. **[4]**

[Total: 7]

SELF-EVALUATION CHECKLIST

After studying this chapter, complete a table like this:

I can	See section...	Needs more work	Almost there	Ready to move on
describe the structure of phospholipids and the fluid mosaic model of membrane structure	4.2			
describe the arrangement of the molecules in cell surface membranes	4.2			
describe the roles of the molecules found in cell surface membranes	4.3			
outline the process of cell signalling	4.4			
explain how substances enter and leave cells across cell surface membranes	4.5			
carry out practical investigations into diffusion and osmosis	4.5			
illustrate the principle that surface area : volume ratios decrease with increasing size	4.5			
explain the movement of water between cells and solutions in terms of water potential	4.5			



Chapter 5
The mitotic cell cycle

LEARNING INTENTIONS

In this chapter you will learn how to:

- describe the structure of chromosomes
- outline the cell cycle – the cycle of events by which body cells grow to a certain size and then divide into two
- describe the behaviour of chromosomes during mitosis and the associated behaviour of the nuclear envelope, the cell surface membrane and the spindle
- identify stages of mitosis in photomicrographs, diagrams and microscope slides
- explain the importance of mitosis
- outline the role of telomeres
- outline the role of stem cells
- explain how uncontrolled cell division can lead to cancer.

BEFORE YOU START

During growth of multicellular organisms, the nucleus divides before the cell divides so that each new cell contains an identical nucleus. With a partner, discuss briefly why this is important. Then carry out the following exercise.

- Make a list of **four** structural features of the nucleus of eukaryotes.
- For each feature, outline its function (or an example of its function).

WHY GROW OLD?

Is it useful to prolong human life? The forerunners of modern chemists, the alchemists, thought so (Figure 5.1). They had two main aims:

- to discover how to transform 'base' metals (e.g. lead) into 'noble' metals (e.g. gold and silver)
- to discover the elixir of life, which would give eternal youth.

By the early 20th century, scientists had relegated these aims to impossible dreams. Now, however, humans are once again challenging the idea that the process of ageing is inevitable.

Why do organisms grow old and die? Interest in the process of ageing was rekindled with the discovery of telomeres in 1978. Telomeres are protective sequences of nucleotides found at the ends of chromosomes, which become shorter every time a cell divides. A gradual degeneration of the organism occurs, resulting in ageing.

Some cells are able to replenish their telomeres using the enzyme telomerase. It is thought that cancer cells can do this and so remain immortal (will never die). It may therefore be possible to prevent the ageing of normal cells by keeping the enzyme telomerase active.

Figure 5.1: A 19th-century oil painting showing an alchemist at work.

Question for discussion

If the ageing process could be slowed or prevented, this would raise some important moral and ethical issues. Try to identify and discuss some of these issues.

5.1 Growth and reproduction

All living organisms grow and reproduce. Living organisms are made of cells, so this means that cells must be able to grow and reproduce. Cells reproduce by dividing and passing on copies of their genes to 'daughter' cells. The process must be very precisely controlled so that no vital genetic information is lost.

In Chapter 6 you will learn how DNA can copy itself accurately. In this chapter you will learn how whole cells can do the same.

In Chapter 1 you saw that one of the most easily recognised structures in eukaryotic cells is the nucleus. The importance of the nucleus has been obvious ever since it was realised that the nucleus always divides before a cell divides. Each of the two daughter cells therefore contains its own nucleus. This is important because the nucleus controls the cell's activities. It does

this because it contains the genetic material, DNA, which acts as a set of instructions, or code, for life (Chapter 6).

All the cells in the bodies of multicellular organisms are genetically identical, apart from the reproductive cells known as gametes. This is because they all come from one cell, the zygote. This is the cell formed when one gamete from your mother and one gamete from your father fused. When the zygote starts the process of growth, it divides into two cells with identical nuclei. This involves a type of nuclear division called mitosis. This process of nuclear division followed by cell division continues to be repeated in a cycle called the mitotic cell cycle to produce all the cells of your body, about 30 trillion in an average human.

You will study the process of mitosis and the mitotic cell cycle in this chapter.

5.2 Chromosomes

Just before a eukaryotic cell divides, a number of threadlike structures called chromosomes gradually become visible in the nucleus. They are easily seen, because they stain intensely with particular stains.

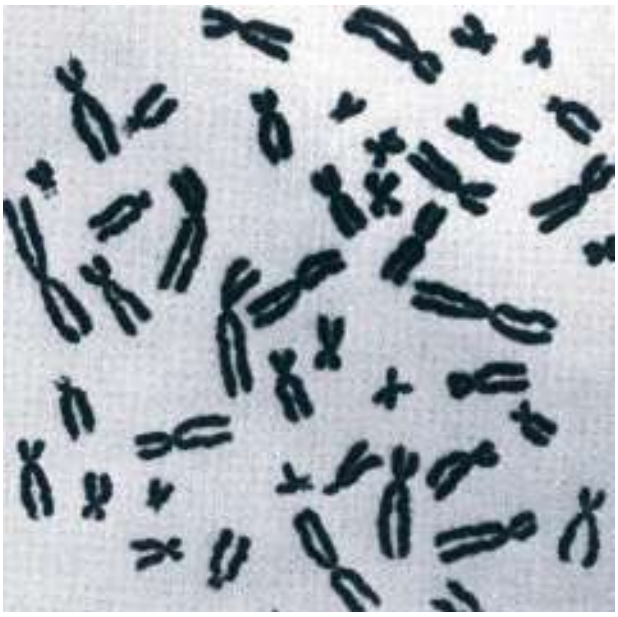


Figure 5.2: Photograph of a set of chromosomes in a human male, just before cell division. Each chromosome is composed of two chromatids held together at the centromere. Note the different sizes of the chromosomes and the different positions of the centromeres.

Before their function was known, they were called chromosomes because 'chromo' means coloured and 'somes' means bodies.

The number of chromosomes is characteristic of the species. For example, in human cells there are 46 chromosomes; in fruit fly cells there are only 8 chromosomes. Figure 5.2 is a photograph of a set of chromosomes in the nucleus of a human cell.

The structure of chromosomes

Before studying nuclear division, you need to understand a little about the structure of chromosomes. Figure 5.3 is a simplified diagram of the structure of a chromosome just before cell division.

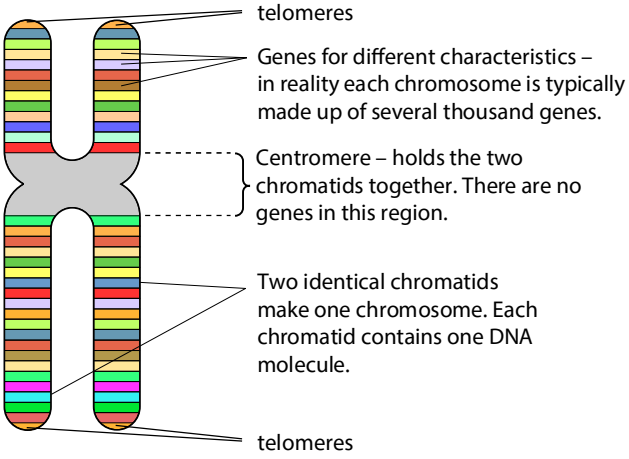


Figure 5.3: Simplified diagram of the structure of a chromosome.

You can see that the chromosome at this stage is a double structure. It is made of two identical structures called **chromatids**, joined together. The two identical chromatids of one chromosome are known as sister chromatids. There are two chromatids because, during the period between nuclear divisions, known as interphase, each DNA molecule in a nucleus makes an identical copy of itself (Chapter 6, Section 6.3, DNA

KEY WORD

chromatid: one of two identical parts of a chromosome, held together by a centromere, formed during interphase by the replication of the DNA strand

replication). Each chromatid contains one of these DNA copies. The sister chromatids are held together by a narrow region called the centromere, to form a single chromosome. The centromere could be anywhere along the length of the chromosome, but the position is characteristic for a particular chromosome.

DNA is the molecule of inheritance and is made up of a series of genes. Each gene is one unit of inheritance. The two DNA molecules, one in each of the sister chromatids, are identical. This means the genes on the chromatids are also identical. The fact that there are two identical chromatids is the key to precise nuclear division. When cells divide, one chromatid goes into one daughter cell and one goes into the other daughter cell, making the daughter cells genetically identical.

So much information is stored in DNA that it needs to be a very long molecule. Although the molecule is only 2 nm wide, the total length of DNA in the 46 chromosomes of an adult human cell is about 1.8 metres. This has to be packed into a nucleus which is only 6 µm in diameter. This is the equivalent of trying to get an 18 km length of string into a ball which is only 6 cm in diameter! A precise scaffolding made of protein molecules prevents the DNA from getting tangled up into knots. The DNA is wound around the outside of these protein molecules. The combination of DNA and proteins is called chromatin. Chromosomes are made of chromatin. Chemically speaking, most of the proteins are basic (the opposite of acidic) and are of a type known as histones. Because they are basic, they can interact easily with DNA, which is acidic.

Chromosomes also possess two more features essential for successful nuclear division: centromeres and telomeres. Centromeres are visible in Figures 5.2 and 5.3. Telomeres are visible if chromosomes are stained appropriately (Figure 5.4). Centromeres are discussed with mitosis in Section 5.4 and the role of telomeres is discussed in Section 5.5.

Question

1 The primary structure of histone protein molecules is highly conserved during evolution, meaning there are extremely few changes over time (far fewer than is usual for proteins).

   a State what is meant by the primary structure of a protein.

   b What does the fact that histone molecules are highly conserved suggest about their functioning?

Histones help to package DNA into a smaller space. The packing ratio is a useful measure of the degree of compactness achieved. If a 10 cm long piece of string was packed into a 5 cm long tube, the packing ratio would be 2 (2 cm of string per cm of tube). The same idea can be applied to the problem of packing DNA into chromosomes.

   c Chromosomes vary in length. A chromosome 10 µm long was estimated to contain 8.7 cm of DNA. What is the packing ratio of DNA in this chromosome? Show your working.

   d There are 46 chromosomes in an adult human cell. Their average length is about 6 µm. The total length of DNA in the 46 chromosomes is about 1.8 m. What is the approximate overall packing ratio for DNA in human chromosomes? Show your working.

   e Explain briefly how histone proteins contribute to reducing the packing ratio for DNA.

5.3 The cell cycle

Mitosis is nuclear division that produces two genetically identical daughter nuclei, each containing the same number of chromosomes as the parent nucleus. Mitosis is part of a precisely controlled process called the **cell cycle**.

The cell cycle is the sequence of events that takes place between one cell division and the next. It has three phases: interphase, nuclear division and cell division. These are shown in Figure 5.5.

During interphase, the cell grows to its normal size after cell division and carries out its normal functions. At some point during interphase, a signal may be received that the cell should divide again. If this happens, the DNA in the nucleus replicates so that each chromosome consists of two identical chromatids. This phase of the cell cycle is called the S phase – S stands for synthesis (of DNA). This is a relatively short phase. The gap after cell division and before the S phase is called the

KEY WORDS

mitosis: the division of a nucleus into two so that the two daughter cells have exactly the same number and type of chromosomes as the parent cell

cell cycle: the sequence of events that takes place from one cell division until the next; it is made up of interphase, mitosis and cytokinesis

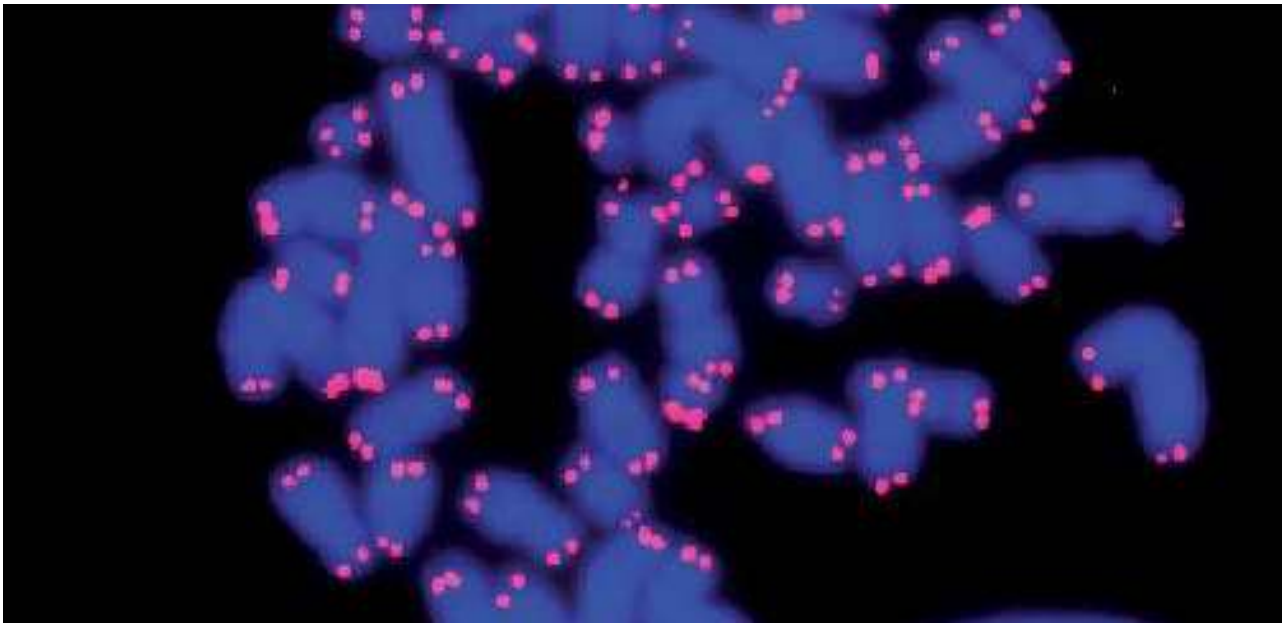


Figure 5.4: Fluorescent staining of human chromosome telomeres as seen with a light microscope. Chromosomes appear blue and telomeres appear pink (×4000).

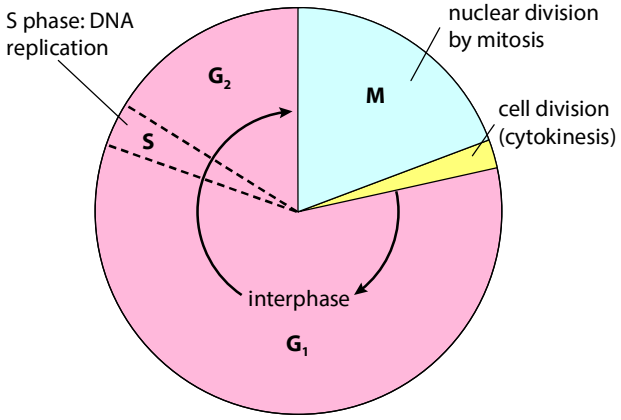


Figure 5.5: The mitotic cell cycle. DNA replication takes place during interphase, the period between cell division and the next nuclear division: **S** = synthesis (of DNA); **G** = gap; **M** = mitosis.

G_1 phase (G for gap). The gap after the S phase and before cell division is called the G_2 phase. Interphase therefore consists of G_1, S and G_2. During G_1, cells make the RNA, enzymes and other proteins needed for growth. At the end of G_1, the cell becomes committed to dividing or not dividing.

During G_2, the cell continues to grow and the new DNA that was made during the S phase is checked. Any errors are usually repaired. Preparations are also made to begin the process of division. For example, there is a sharp increase in production of the protein tubulin which is needed to make microtubules for the mitotic spindle.

Nuclear division follows interphase. Nuclear division is referred to as the M phase (M for mitosis). Growth stops temporarily during mitosis. After the M phase, when the nucleus has divided into two, the whole cell divides to create two genetically identical cells. In animal cells, cell division involves constriction of the cytoplasm between the two new nuclei, a process called cytokinesis. In plant cells, it involves the formation of a new cell wall between the two new nuclei.

The length of the cell cycle is very variable, depending on environmental conditions and cell type. On average, root tip cells of onions divide once every 20 hours; epithelial cells in the human intestine every 10 hours.

5.4 Mitosis

The process of mitosis is best described by annotated diagrams as shown in Figure 5.6. Although in reality the process is continuous, it is usual to divide it into four main stages for convenience, like four snapshots from a film. The four stages are called prophase, metaphase, anaphase and telophase.

Most nuclei contain many chromosomes, but the diagrams in Figure 5.6 show a cell containing only four chromosomes for convenience. Colours are used to show whether the chromosomes are from the female or male parent. An animal cell is used as an example. Note that during late prophase the nuclear envelope 'disappears'. In fact, it breaks up into small vesicles which cannot be seen with a light microscope. It reassembles during telophase, as shown in Figure 5.6. As a result, diagrams of metaphase and anaphase do not show the nuclear envelope. At the end of telophase, after the nucleus has divided, the cell divides by constriction of the cytoplasm, a process called cytokinesis. As the cell changes shape, the surface area of the cell increases as the two new cells form, so new cell surface membrane has to be made.

The behaviour of chromosomes in plant cells is identical to that in animal cells. However, plant cells differ in two ways:

- plant cells do not contain centrosomes
- after nuclear division of a plant cell, a new cell wall must form between the daughter nuclei.

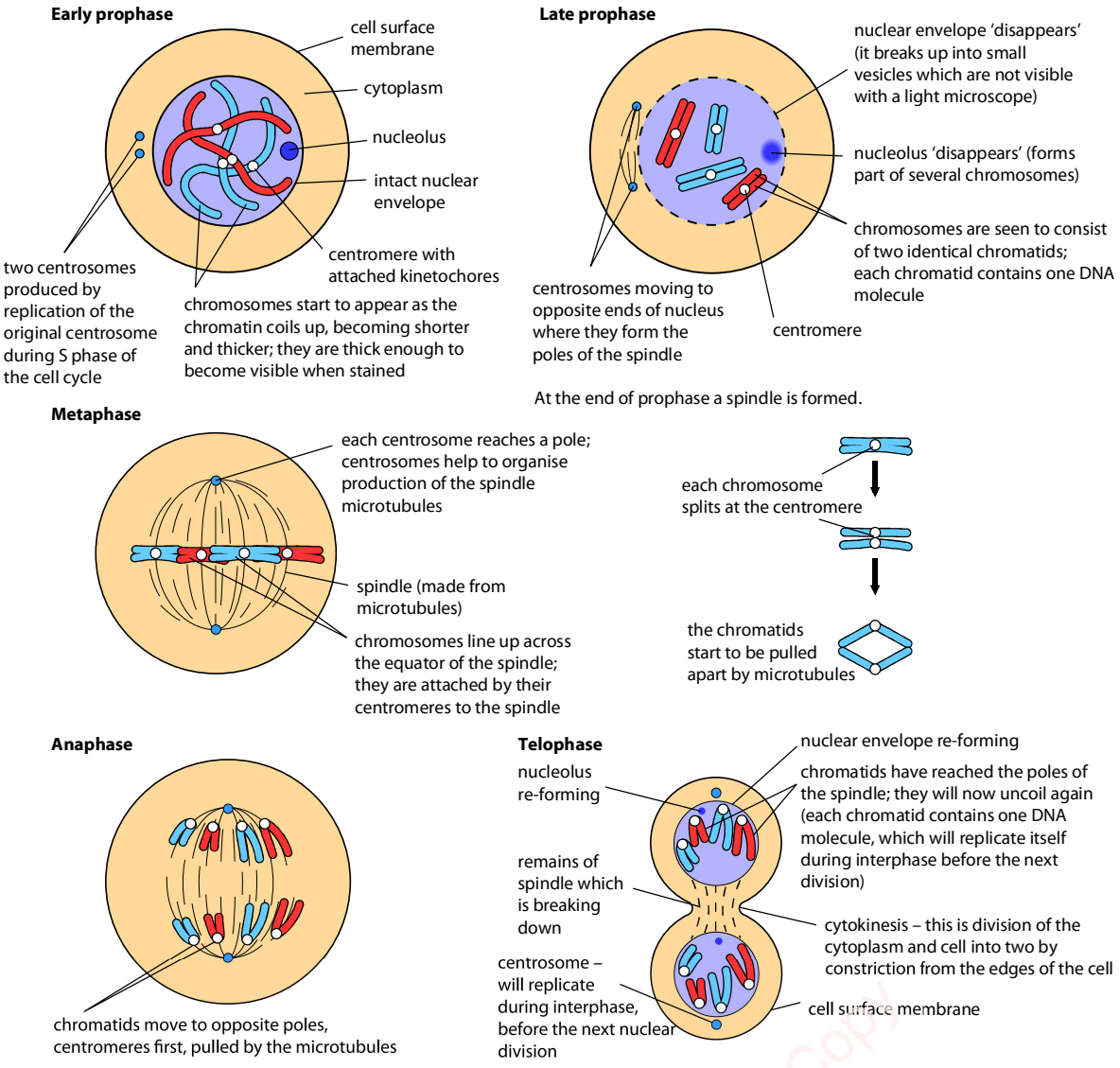
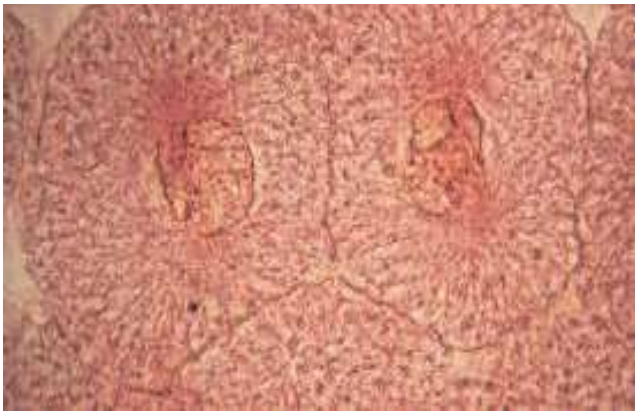
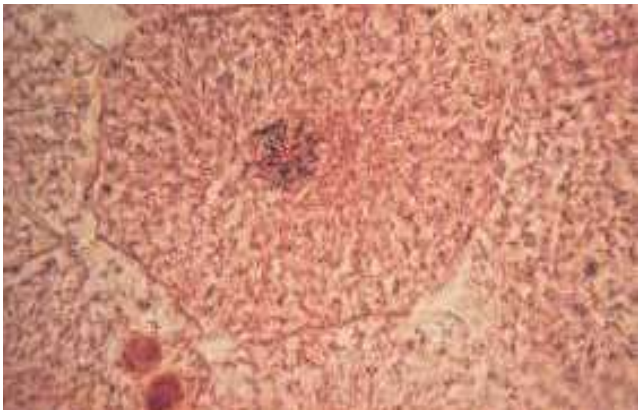


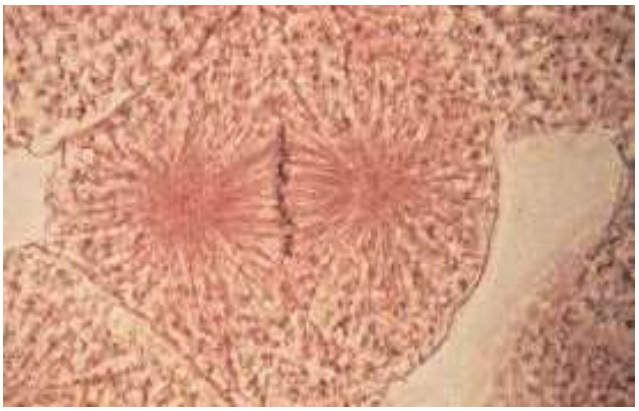
Figure 5.6: Mitosis and cytokinesis in an animal cell.



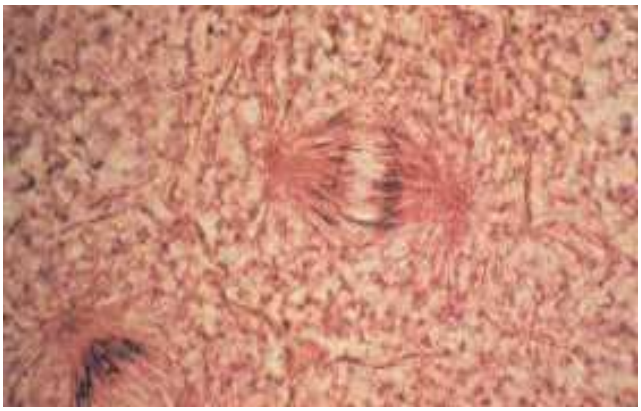
a Prophase.



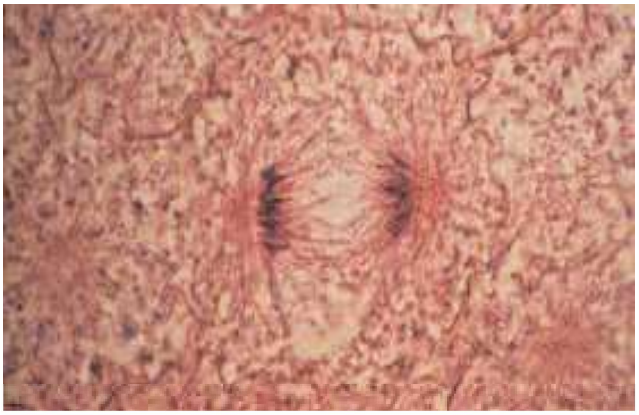
b Stage intermediate between prophase and metaphase.



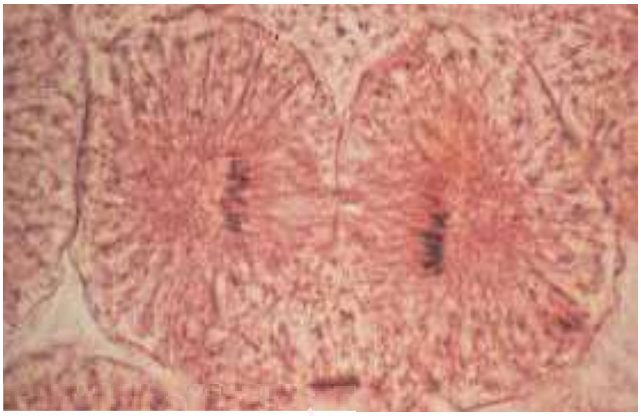
c Metaphase: the spindle fibres (microtubules) are now clearly visible, and the centrosomes are located at opposite ends of the spindle in the centre of a star-shaped arrangement of radiating microtubules.



d Early anaphase.



e Anaphase.



f Telophase and cell division (cytokinesis).

Figure 5.7: Stages of mitosis and cell division in an animal cell (whitefish) (×900). Chromosomes are stained darkly.

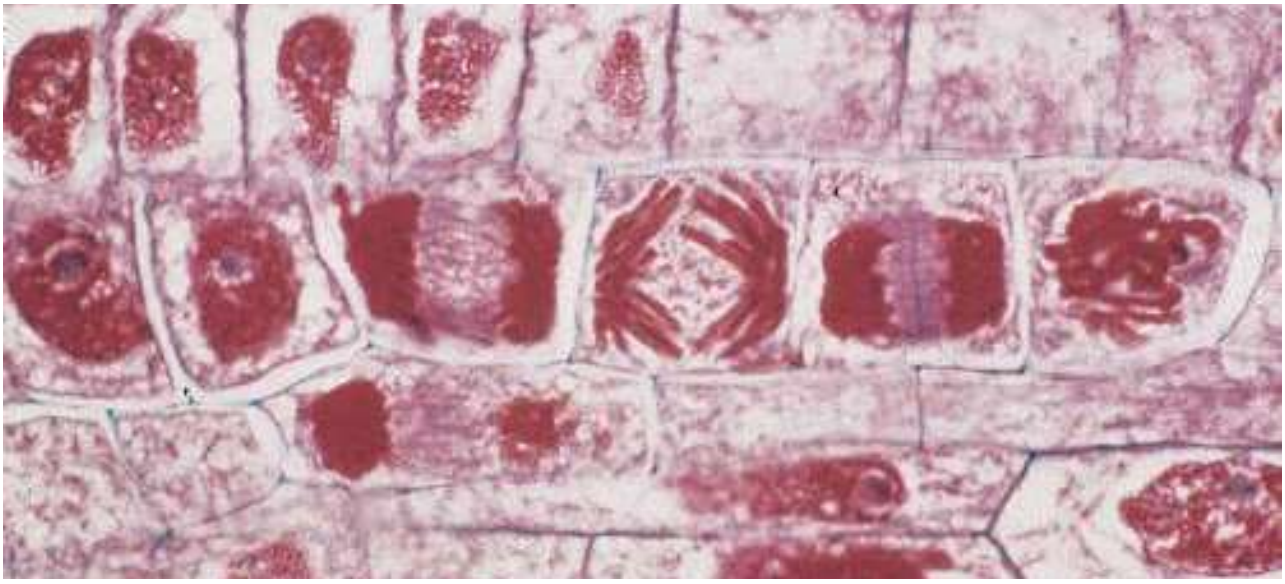


Figure 5.8: Longitudinal section (LS) of onion root tip showing stages of mitosis and cell division typical of plant cells (×400). Try to identify the stages based on information given in Figure 5.7.

It is the behaviour of the chromosomes, though, that is of particular interest. Figure 5.7 (animal) and Figure 5.8 (plant) show photographs of mitosis as seen with a light microscope.

Centrosomes, centrioles and centromeres

Centrosomes are located at the poles of the spindle, one at each pole. (The poles are the two ends of the spindle. The spindle gets its name from the fact that it is similar in shape to some spindles used in spinning – Sleeping Beauty pricked her finger on a spindle in the well-known fairy tale.) As noted in Chapter 1, the centrosome is an organelle found in animal cells that acts as a microtubule organising centre (MTOC). Centrosomes are responsible for making the spindle, which is made of microtubules. The spindle is needed for separation of the chromatids. Each centrosome consists of a pair of centrioles surrounded by a large number of proteins. It is these proteins that control production of the microtubules, not the centrioles. Plant mitosis occurs without centrosomes.

The centromere holds the chromatids together (see Figures 5.2 and 5.3), but is also involved in the separation of chromatids during mitosis. During mitosis the centromere is the site of attachment of spindle microtubules. Each metaphase chromosome has two **kinetochores** at its centromere, one on each chromatid (Figure 5.9).

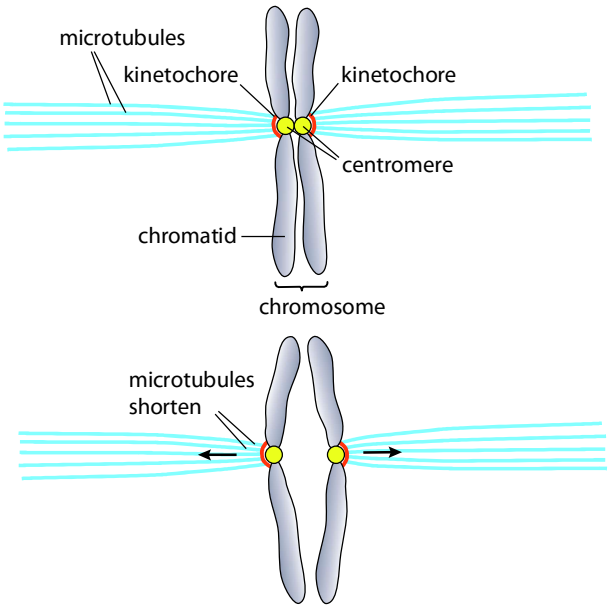


Figure 5.9: Role of the centromere, kinetochores and microtubules during mitosis

KEY WORD

kinetochore: a protein structure found at the centromere of a chromatid to which microtubules attach during nuclear division

The kinetochores are made of protein molecules which connect the centromere to the spindle microtubules. Bundles of microtubules called spindle fibres extend from the kinetochores to the poles of the spindle during mitosis. Construction of kinetochores begins before nuclear division starts (during the S phase of the cell cycle) and they are lost again afterwards.

The microtubules attached to the kinetochore pull the kinetochore towards the pole of the spindle. The rest of the chromatid drags behind, giving the characteristic > or < shape of chromatids during anaphase (Figures 5.6–5.8). The pulling action is achieved by shortening of the microtubules, both from the pole end and from the kinetochore end.

Question

2 How can the microtubules be shortened? (Refer back to Chapter 1.)

Importance of mitosis

Growth of multicellular organisms

The two daughter cells formed after mitosis have the same number of chromosomes as the parent cell and are genetically identical (that is, they are clones). This allows growth of multicellular organisms from unicellular zygotes. Growth may occur over the entire body, as in animals, or be confined to certain regions, as in the meristems (growing points) of plants.

Replacement of damaged or dead cells and repair of tissues by cell replacement

This is possible using mitosis followed by cell division. Cells are constantly dying and being replaced by identical cells. In the human body, for example, cell replacement is particularly rapid in the skin and in the lining of the gut. Some animals are able to regenerate whole parts of the body; for example, starfish can regenerate new arms.

Asexual reproduction

Mitosis is the basis of **asexual reproduction**, the production of new individuals of a species by a single parent organism. The offspring are genetically identical to the parents. Asexual reproduction can take many forms.

KEY WORD

asexual reproduction: the production of new individuals of a species by a single parent organism

For a unicellular organism such as *Amoeba,* cell division inevitably results in reproduction. For multicellular organisms, new individuals may be produced which bud off from the parent in various ways (Figure 5.10). Budding is particularly common in plants. It is most commonly a form of vegetative propagation in which a bud on part of the stem simply grows a new plant. The new plant eventually becomes detached from the parent and lives independently. The bud may be part of the stem of an overwintering structure such as a bulb or tuber. The ability to generate whole organisms from single cells or small groups of cells is important in biotechnology and genetic engineering, and it is the basis of cloning.

Figure 5.10: a Asexual reproduction by budding in *Hydra* (×60). *Hydra* lives in fresh water, catching its prey with the aid of its tentacles. The bud growing from its side is genetically identical to the parent and will eventually break free and live independently. **b** Asexual reproduction in *Kalanchoe pinnata*. The plant produces genetically identical new individuals along the edges of its leaves.

Immune response

The cloning of B- and T-lymphocytes during the immune response is dependent on mitosis (Chapter 11, Section 11.2, Cells of the immune system).

Questions

3 Outline how mitosis allows asexual reproduction to take place.

4 Human cells contain 46 chromosomes. In the mitotic cell cycle of a human cell:
   - a how many chromatids are present as the cell enters mitosis?
   - b how many DNA molecules are present?
   - c how many kinetochores are present?
   - d how many chromatids are present in the nucleus of each daughter cell after mitosis and cell division?
   - e how many chromatids are present in the nucleus of a cell after replication of DNA?

5 Draw a simple diagram of a cell which contains only one pair of chromosomes:
   - a at metaphase of mitosis
   - b at anaphase of mitosis.

6 State **two** functions of centromeres during nuclear division.

7 Thin sections of adult mouse liver were prepared and the cells stained to show up the chromosomes. In a sample of 75 000 cells examined, 9 were found to be in the process of mitosis. Calculate the length of the cell cycle in days in mouse liver cells, assuming that mitosis lasts one hour.

PRACTICAL ACTIVITY 5.1

Investigating mitosis using a root tip squash

Growth in plants is confined to regions known as meristems. A convenient example to study is the root tip meristem. This lies just behind the protective root cap. In this meristem there is a zone of cell division containing small cells in the process of mitosis.

You may be able to study commercially prepared permanent slides of root tips. You can also make your own temporary slides. Cutting thin sections of plant material is tricky, but this is not needed if the squash technique is used. This involves staining the root tip, then gently squashing it. This spreads the cells out into a thin sheet in which individual dividing cells can be clearly seen.

Procedure

The root tips of garlic, onion, broad bean and sunflower provide suitable material. Bulbs or seeds can be grown suspended by a pin over water for a period of a week or two. The tips of the roots (about 1 cm) are removed and placed in a suitable stain such as warm, acidified acetic orcein. This stains chromosomes a deep purple. The stained root tip can be squashed into a sheet of cells on a glass slide, using a blunt instrument such as the end of the handle of a mounted needle.

You should be able to see and draw cells similar to those shown in Figure 5.8 (but note that Figure 5.8 shows a longitudinal section of a root tip, not a squash). You could also use Figure 5.8 to make some annotated drawings of the different stages of mitosis.

(See Practical Investigation 5.1 in the Practical Workbook for additional information.)

5.5 The role of telomeres

You have seen that DNA is replicated (copied) during the S phase of the cell cycle. The copying enzyme cannot run to the end of a strand of DNA and complete the replication – it stops a little short of the end. (It is not possible to understand the reason for this without a detailed knowledge of replication.) If part of the DNA is not copied, that piece of information is lost. At each subsequent division, another small section of information from the end of the DNA strand would be lost. Eventually, the loss of vital genes would result in cell death.

The main function of **telomeres** is to ensure that the ends of the molecule are included in the replication and not left out when DNA is replicated. Telomeres are found at the ends of chromosomes (see Figure 5.11 and also Figure 5.4). They have been compared with the plastic tips on the ends of shoe laces. Telomeres are made of DNA with short base sequences that are repeated many times ('multiple repeat sequences').

Telomeres work by making the DNA a bit longer. They have no useful information, but allow the copying enzyme to complete copying all the meaningful DNA. As long as extra bases are added to the telomere during each cell cycle to replace those that are not copied, no vital information will be lost from the non-telomere DNA and the cell will be able to continue dividing successfully. The enzyme that performs the role of adding bases to telomeres is called telomerase. The main function of telomeres is therefore to prevent the loss of genes during cell division and to allow continued replication of a cell.

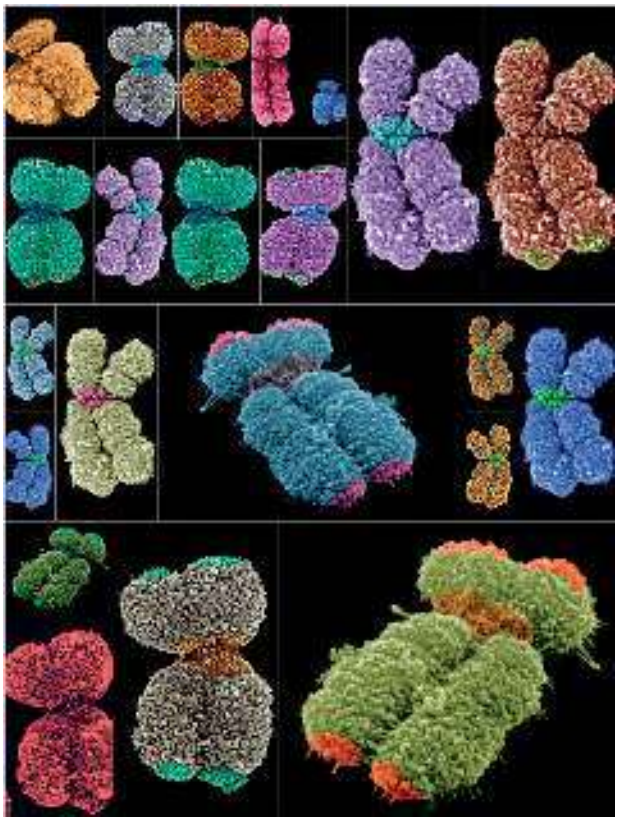


Figure 5.11: Coloured scanning electron micrographs of human chromosomes showing the location of telomeres at the ends of the chromosomes. Chromatids and centromeres are also clearly visible. Telomeres contain short repeated sequences of DNA. As cells replicate and age, the telomeres gradually get shorter. Stem cells are an exception.

Some cells do not 'top up' their telomeres at each division. These tend to be fully differentiated (specialised) cells. With each division, their telomeres get a little shorter until the vital DNA is no longer protected and the cell dies. This could be one of the mechanisms of ageing, by which humans grow old and die. This, of course, suggests that by somehow preventing the loss of telomeres scientists might be able to slow down or even prevent the process of ageing (see 'Why grow old?' at the beginning of the chapter).

5.6 The role of stem cells

A **stem cell** is a cell that can divide an unlimited number of times (by mitosis). When it divides, each new cell has the potential to remain a stem cell or to develop (differentiate) into a specialised cell such as a blood cell or a muscle cell.

The power of a stem cell to produce different types of cell is variable and is referred to as its potency. Stem cells that can produce any type of cell are described as totipotent. The zygote formed by the fusion of a sperm with an egg at fertilisation is totipotent, as are all the cells up to the 16-cell stage of development in humans. After that, some cells become specialised to form the placenta, while others lose this ability but can form all the cells that will lead to the development of the embryo and later the adult. These embryonic stem cells are described as pluripotent.

As tissues, organs and systems develop, cells become more and more specialised. There are more than 200 different types of cell in an adult human body.

KEY WORDS

telomere: repetitive sequence of DNA at the end of a chromosome that protects genes from the chromosome shortening that happens at each cell division

stem cell: a relatively unspecialised cell that retains the ability to divide an unlimited number of times, and which has the potential to become a specialised cell (such as a blood cell or muscle cell)

Question

8 As a result of mitosis, all 200+ different types of cell contain the same set of genes as the zygote. Genes control the activities of cells. What does this suggest about the mechanism by which cells become different?

The more 'committed' cells become to particular roles, the more they lose the ability to divide until, in the adult, most cells do not divide. However, for growth and repair it is essential that small populations of stem cells remain which can produce new cells. Adult stem cells have already lost some of the potency associated with embryonic stem cells and are no longer pluripotent. They are only able to produce a few types of cell and may be described as multipotent. For example, the stem cells found in bone marrow are of this type. They can replicate any number of times, but can produce only blood cells, such as red blood cells, monocytes, neutrophils and lymphocytes. Mature blood cells have a relatively short lifespan, so the existence of these stem cells is essential. For example, around 250 billion red blood cells and 20 billion white blood cells are lost and must be replaced each day.

In the adult, stem cells are found throughout the body – for example, in the bone marrow, skin, gut, heart and brain. Research into stem cells has opened up some exciting medical applications. Stem cell therapy is the introduction of new adult stem cells into damaged tissue to treat disease or injury. Bone marrow transplantation is an example of this therapy that has progressed beyond the experimental stage into routine medical practice. It is used to treat blood and bone marrow diseases, and blood cancers such as leukaemia. In the future, it is hoped to be able to treat conditions such as diabetes, muscle and nerve damage, and brain disorders such as Parkinson's and Huntington's diseases. Experiments with growing new tissues, or even organs, from isolated stem cells in the laboratory have also been conducted.

5.7 Cancers

In high-income countries, **cancers** cause roughly one in four deaths. Globally, cancers account for about one in six deaths (9.6 million people in 2018). This makes cancers second only to cardiovascular disease as a cause of death. There are more than 200 different forms of cancer, and the medical profession no longer thinks of cancers as a single disease.

Cancers illustrate the importance of controlling cell division precisely, because cancers are a result of uncontrolled mitosis. Cancerous cells divide repeatedly and form a tumour, which is an irregular mass of cells. Figure 5.12 shows a tumour in the lung of a patient who died of lung cancer compared to a healthy lung (from a patient who died from some other cause). Worldwide, lung cancer kills more people than any other cancer. Cancer cells usually show abnormal changes in shape (Figure 5.13).

Cancers start when changes occur in the genes that control cell division. A change in any gene is called a **mutation**. The term for a mutated gene that causes cancer is an *oncogene*, from the Greek word 'onkos' meaning bulk or mass. Mutations causing cancer can be inherited but most of the mutations that cause cancers occur over the course of the lifetime of an individual. Mutations are not unusual events, and most of the time they do not lead to cancer. Most mutated cells are affected in some way that results in their early death or their destruction by the body's immune system. Most cells can be replaced, so mutation usually has no harmful effect on the body. Unfortunately, cancer cells manage to escape both cell death and destruction so, although the mutation may originally occur in only one cell, it is passed on to all that cell's descendants. By the time it is detected, a typical tumour usually contains about a billion cancer cells. Any agent, such as asbestos, that causes cancer is called a **carcinogen** and is described as carcinogenic.

Although you do not need to know about different types of tumour, you may be interested to know that not all tumours are cancerous. Some tumours do not spread from their site of origin – these are known as

KEY WORDS

cancers: a group of diseases that result from a breakdown in the usual control mechanisms that regulate cell division; certain cells divide uncontrollably and form tumours, from which cells may break away and form secondary tumours in other areas of the body (metastasis)

mutation: a random change in the base sequence (structure) of DNA (a gene mutation), or in the structure and/or number of chromosomes (a chromosome mutation)

carcinogen: a substance or environmental factor that can cause cancer

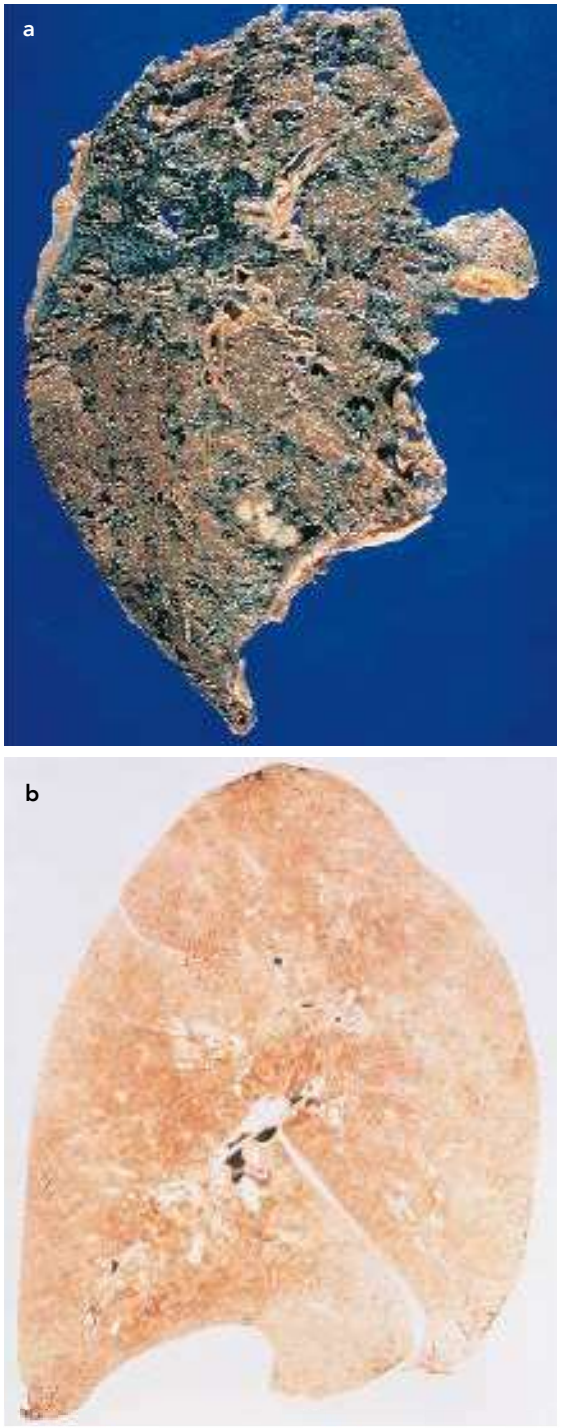


Figure 5.12: a Lung of a patient who died of lung cancer, showing rounded deposits of tumour (white area at bottom of picture). Black tarry deposits throughout the lung show the patient was a heavy smoker. **b** Section of a healthy human lung. No black tar deposits are visible.

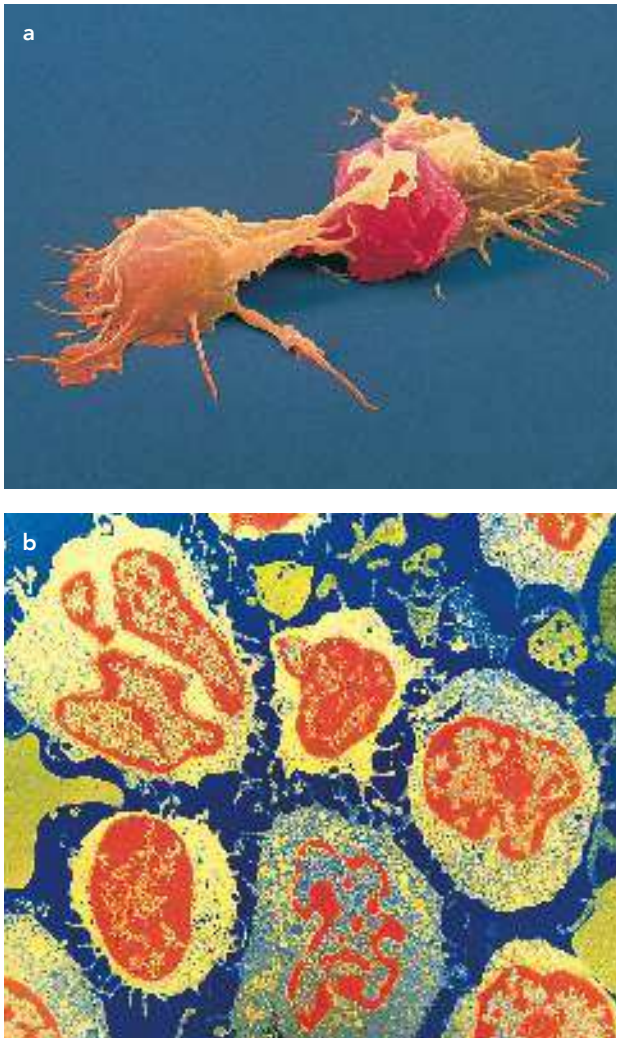


Figure 5.13: a False-colour scanning electron micrograph of a cancer cell (red) and white blood cells (orange and yellow). White blood cells gather at cancer sites as an immune response. They are beginning to flow around the cancer cell, which they will kill using toxic chemicals (×4500). **b** False-colour transmission electron micrograph (TEM) of abnormal white blood cells isolated from the blood of a person with hairy-cell leukaemia. The white blood cells are covered with characteristic hair-like cytoplasmic projections. Leukaemia is a disease in which the bone marrow and other blood-forming organs produce too many of certain types of white blood cells. These immature or abnormal cells suppress the normal production of white and red blood cells, and increase the patient's susceptibility to infection (×6400).

benign tumours; warts are a good example. It is only tumours that spread through the body, invading and destroying other tissues, that cause cancer. These are known as malignant tumours. Malignant tumours interfere with the normal functioning of the area where they have started to grow. They may block the intestines, lungs or blood vessels. Cells can break off and spread through the blood and lymphatic system to other parts of the body to form secondary growths. The spread of cancers in this way is called metastasis. It is the most dangerous characteristic of cancer because it can be very hard to find the secondary cancers and remove them.

The steps involved in the development of cancer are shown in Figure 5.14.

Question

9 Research is being carried out into ways of inactivating the enzyme telomerase in cancer cells. Explain the reason for this.

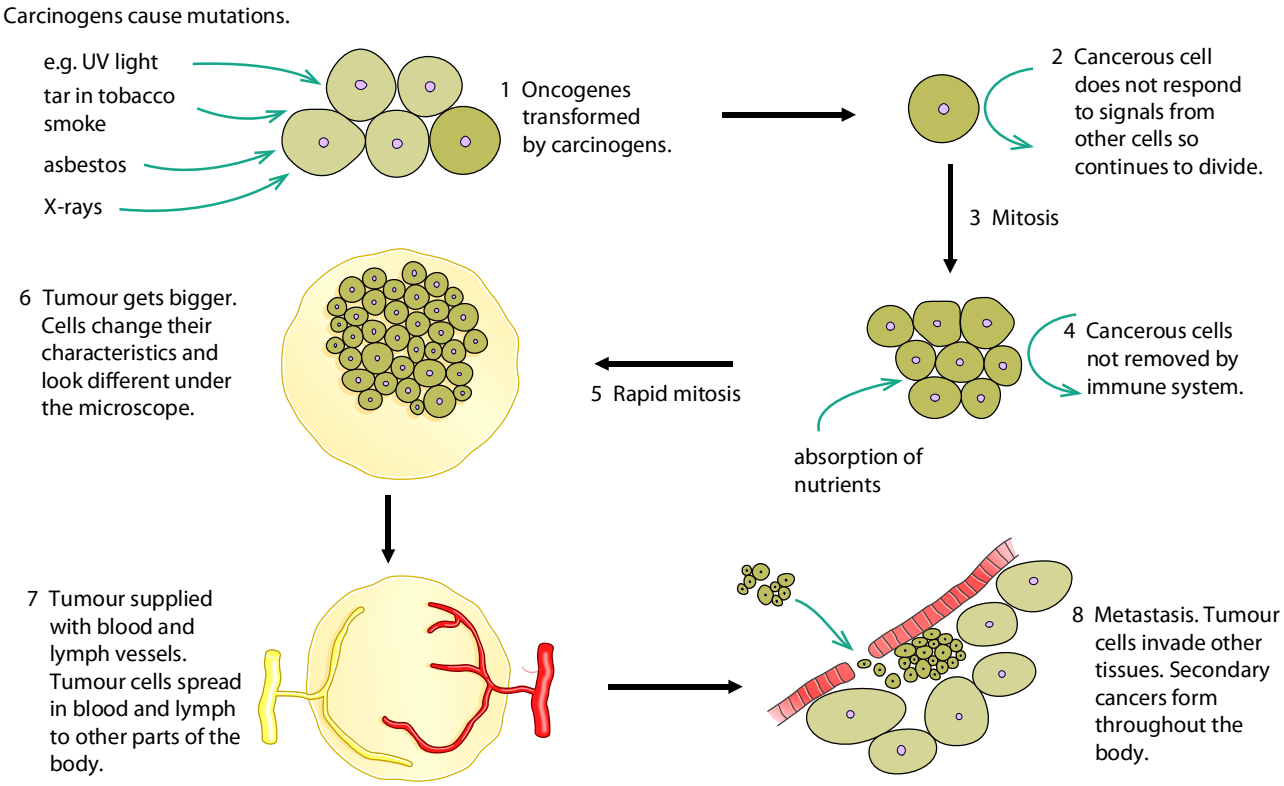


Figure 5.14: Stages in the development of cancer.

REFLECTION

Make a set of two pairs of chromosomes (one short pair, one long pair so they can easily be distinguished) as in Figure 5.6 late prophase. Use pipe-cleaners or Pop or Poppit beads which can be joined together to represent chromatids. Use two different colours if possible (to represent their origins from male and female parents), though this is not essential.

Use these model chromosomes to test your understanding of the stages of mitosis. It is useful to draw a large spindle on a large sheet of paper on which the model chromosomes can be moved appropriately.

CONTINUED

Personal reflection question

What did you enjoy about this activity? What parts of it did you particularly like or dislike? Why? Will it help you to remember the process of mitosis?

Final reflection

Discuss with a friend which, if any, parts of Chapter 5 you need to:

- read through again to make sure you really understand
- seek more guidance on, even after going over it again.

SUMMARY

Chromosomes are made of chromatin. Chromatin consists mainly of DNA wrapped around basic protein molecules called histones.
During nuclear division chromosomes become visible and are seen to consist of two chromatids held together by a centromere. Each chromatid contains one DNA molecule.
Growth of a multicellular organism is a result of cells dividing to produce genetically identical daughter cells.
During cell division, the nucleus divides first, followed by division of the whole cell. Division of a nucleus to produce two genetically identical nuclei is achieved by the process of mitosis. Mitosis is divided into four phases: prophase, metaphase, anaphase and telophase.
Mitosis is used in growth, repair, asexual reproduction and cloning of cells during an immune response.
The period from one cell division to the next is called the cell cycle. It has four phases: G_1 is the first growth phase after cell division; S phase is when the DNA replicates; G_2 is a second growth phase; M phase is when nuclear division takes place (followed by cell division).
The ends of chromosomes are capped with special regions of DNA known as telomeres. Telomeres are needed to prevent the loss of genes from the ends of chromosomes during replication of DNA.
Many specialised cells lose the ability to divide, but certain cells known as stem cells retain this ability. Stem cells are essential for growth from zygote to adult and for cell replacement and tissue repair in the adult.
The behaviour of chromosomes during mitosis can be observed in stained preparations of root tips, either in section or in squashes of whole root tips.
Cancers are tumours resulting from repeated and uncontrolled mitosis. They are thought to start as the result of mutation.

EXAM-STYLE QUESTIONS

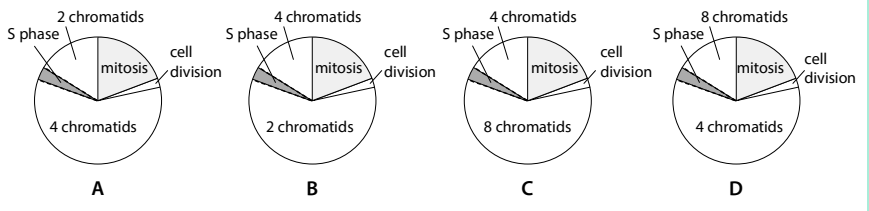
1 During prophase of mitosis, chromosomes consist of two chromatids. At which stage of the cell cycle is the second chromatid made?

A cytokinesis
B G_1
C G_2
D S [1]

2 Growth of cells and their division are balanced during the cell cycle. Which column shows the consequences that would follow from the two errors shown in the table? [1]

Error	Consequence			
	A	B	C	D
speeding up the growth rate without speeding up the cell cycle	larger and larger cells	larger and larger cells	smaller and smaller cells	smaller and smaller cells
speeding up the cell cycle without speeding up the growth rate	larger and larger cells	smaller and smaller cells	larger and larger cells	smaller and smaller cells

3 A cell with four chromosomes undergoes a cell cycle including mitosis. Which diagram correctly shows the changes in chromatid number during interphase?



[1]

4 Cell potency refers to the varying ability of stem cells to:

A create more copies of themselves
B differentiate into different cell types
C produce different types of blood cells
D stimulate growth of tissues [1]

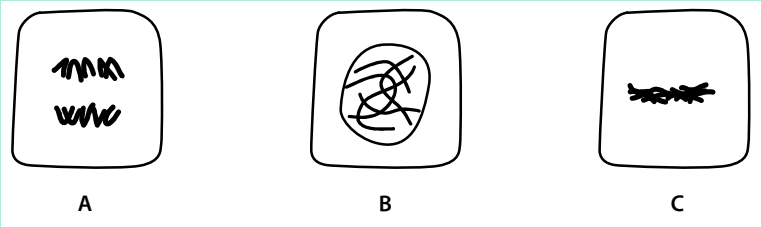
5 Stem cells found in bone marrow are:

A multipotent
B omnipotent
C pluripotent
D totipotent [1]

6 Distinguish between the following terms: centrosome, centriole and centromere. [6]

CONTINUED

7 The diagram shows three cells (labelled **A**, **B** and **C**) from a root tip which has been stained to show chromosomes.

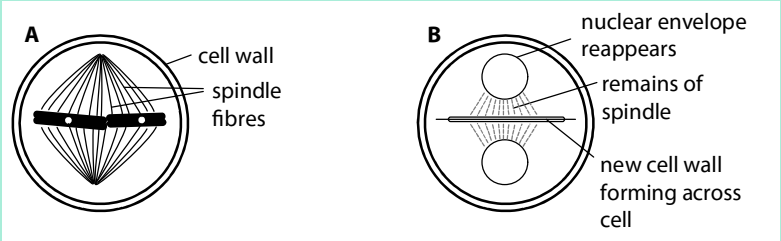


a Identify the stage of mitosis shown by each cell. **[3]**

b Describe what is happening at each stage. **[3]**

[Total: 6]

8 **a** Diagram **A** shows a plant cell dividing by mitosis. Only two chromosomes are shown for simplicity.

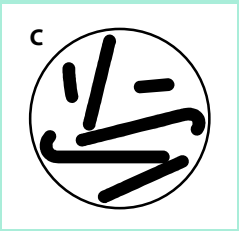


i What stage of mitosis is shown in diagram **A**? **[1]**

ii Draw prophase for the same cell. **[1]**

b Diagram **B** shows the same cell at telophase. The cell is beginning to divide and a new cell wall is forming, spreading out from the middle of the cell. Copy the diagram and add drawings of the chromosomes as they would appear at this stage. **[1]**

c Diagram **C** shows chromosomes in the nucleus of an animal cell.



Draw a diagram to show what the nucleus would look like in anaphase of mitosis. **[3]**

[Total: 6]

CONTINUED

9 In Chapter 1 it was noted that microtubules are tiny tubes made by protein subunits joining together. The protein is called tubulin. Colchicine is a natural chemical which binds to tubulin molecules, preventing the formation of microtubules.

a Why should the binding of colchicine to tubulin molecules interfere with the formation of microtubules? [2]

b What structure or structures involved in mitosis are made of microtubules? [2]

c When cells treated with colchicine are observed, the dividing cells are all seen to be in the same stage of mitosis. Suggest, with reasons, the identity of this stage. [3]

[Total: 7]

10 Which of the following statements are true and which are false?

A Centrosomes are replicated before M phase of the cell cycle begins.

B Sister chromatids contain identical DNA.

C The microtubules attached to a given kinetochore extend to both poles of the spindle.

D Microtubule polymerisation and depolymerisation is a feature of the S phase of the cell cycle.

E Kinetochores are found in the centrosomes.

F Telomeres are the sites of attachment of microtubules during mitosis.

G Sister chromatids remain paired as they line up on the spindle at metaphase.

[1 mark each]

[Total: 7]

11 a Cancer has been described as a genetic disease. Explain why. [2]

b **Define** the term *carcinogen*. [1]

c The diagram on the next page shows the number of people suffering from cancer worldwide, separated into different age categories. It also shows changes between the years 1990 and 2016.

i State the age category in which cancer is most common. [1]

ii Suggest why this age group has the greatest number of cancer cases. [3]

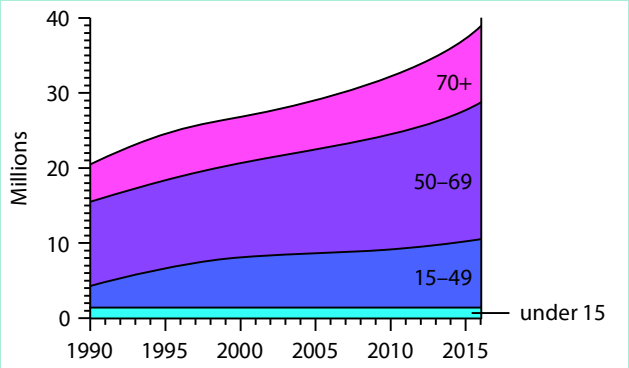
COMMAND WORD

Define: give the precise meaning.

CONTINUED

iii **Comment** on the overall changes shown between 1990 and 2016. **[5]**

[Total: 12]



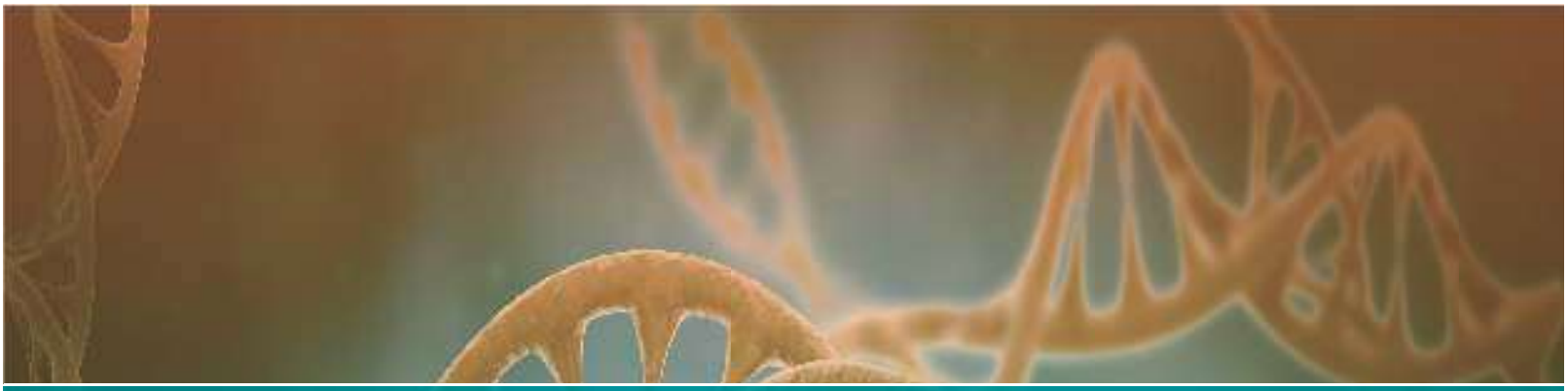
COMMAND WORD

Comment: give an informed opinion.

SELF-EVALUATION CHECKLIST

After studying this chapter, complete a table like this:

I can	See section...	Needs more work	Almost there	Ready to move on
describe the structure of chromosomes	5.2			
outline the cell cycle – the cycle of events by which body cells grow to a certain size and then divide into two	5.3			
describe the behaviour of chromosomes during mitosis and the associated behaviour of the nuclear envelope, the cell surface membrane and the spindle	5.4			
identify stages of mitosis in photomicrographs, diagrams and microscope slides	5.4			
explain the importance of mitosis	5.4			
outline the role of telomeres	5.5			
outline the role of stem cells	5.6			
explain how uncontrolled cell division can lead to cancer	5.7			



Chapter 6

Nucleic acids and protein synthesis

LEARNING INTENTIONS

In this chapter you will learn how to:

- describe the structure of nucleotides, including ATP
- describe briefly the structures of the bases found in DNA and RNA (adenine, guanine, thymine, cytosine and uracil)
- describe the structure of DNA
- describe the structure of RNA, using messenger RNA as an example
- describe the semi-conservative replication of DNA
- explain how the sequence of amino acids in a polypeptide is coded for by a sequence of nucleotides in DNA (a gene)
- describe the principle of the universal genetic code in which DNA bases code for amino acids plus start and stop signals
- explain the roles of DNA and RNA in the transcription and translation stages of protein synthesis
- explain the modification of RNA after transcription and the nature of introns and exons
- explain the nature, types and effects of gene mutations.

BEFORE YOU START

- Where is DNA found in cells?
- Why is DNA sometimes called 'the molecule of life'?
- It can be argued that the discovery of the structure of DNA is one of the most important discoveries ever made by humans. How would you justify this statement?

EDITING HUMANS

The nucleic acid DNA controls growth and development, but it sometimes carries mutations (changes) that can be harmful. There is a long list of human genetic diseases, such as cystic fibrosis and haemophilia, caused by 'faulty' DNA. A dream of the medical profession is to be able to safely correct the faults by the process of gene editing. Gene-editing tools act like molecular scissors, cutting DNA so that a new gene can be inserted or a problem gene can be removed.

Much research has been carried out on animals and tests have also been done on human cells in the laboratory, but in 2017 Brian Madeux, a 44 year old American, became the first human to receive treatment that acts inside the body. Brian suffers from Hunter syndrome. This rare inherited condition causes mucopolysaccharides to build up in body tissues and shortens life expectancy. It is caused by lack of a lysosomal enzyme. The new treatment consisted of introducing into the blood system billions of harmless virus particles which were thereby circulated to vital organs. The viruses carried the DNA for a gene-editing tool and also carried copies of the correct gene. Other sufferers of the syndrome have now received similar treatment and some have begun to show signs of improvement. The technique is still being developed.

Scientists in China currently lead the world in gene-editing techniques. Large-scale trials are underway tackling diseases such as cancer and HIV. The gene-editing tool most commonly used, and used by the Chinese, is known as CRISPR-Cas9. This was invented in 2013 by the American scientist Jennifer Doudna (Figure 6.1).

Figure 6.1: Jennifer Doudna, inventor of the gene editing tool CRISPR-Cas9 which has revolutionised the speed and cost of editing genes.

Despite optimism about future progress, there are strong ethical and moral issues. In 2018 the UK's Nuffield Council on Bioethics agreed that changing the DNA of a human embryo could be 'morally permissible' if it were in the child's best interests, but work on human embryos is still illegal in the UK and many other countries. There are fears about the safety of the treatment as well as issues such as the fact that changes to DNA are permanent, and any long-term effects, which could be passed on to future generations, are unknown.

Question for discussion

Discuss the moral and ethical issues relating to gene editing in human embryos.

6.1 The molecule of life

If you wanted to design a molecule that could act as the genetic material in living things, it would have to have two key features:

- the ability to store information – the information needed is a set of instructions for controlling the behaviour of cells
- the ability to copy itself accurately – whenever a cell divides it must pass on exact copies of the 'genetic molecule' to each of its daughter cells so no information is lost.

Until the mid 1940s, biologists assumed that such a molecule must be a protein. Only proteins were thought to be complex enough to be able to carry the huge number of instructions which would be necessary to make such a complicated structure as a living organism. But during the 1940s and 1950s, evidence came to light that proved beyond doubt that the genetic molecule was not a protein at all, but DNA.

6.2 The structure of DNA and RNA

DNA stands for deoxyribonucleic acid, and RNA stands for ribonucleic acid. As you saw in Chapter 2, DNA and RNA are macromolecules (giant molecules). Together they are known as nucleic acids because they were originally found in the nucleus. Proteins and polysaccharides are also macromolecules. You also saw in Chapter 2 that macromolecules are polymers, made up of many similar, smaller molecules (monomers) joined together to form a long chain. The monomers from which DNA and RNA molecules are made are **nucleotides**. DNA and RNA are therefore **polynucleotides**.

KEY WORDS

nucleotide: a molecule consisting of a nitrogen-containing base, a pentose sugar and a phosphate group

polynucleotide: a chain of nucleotides joined together by phosphodiester bonds

Nucleotides

Nucleotides are made up of three smaller components. These are:

- a nitrogen-containing base
- a pentose sugar
- a phosphate group.

Figure 6.2 shows the structure of a nucleotide and how its three components fit together.

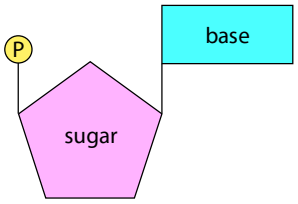


Figure 6.2: Structure of a nucleotide. A nucleotide is made of a nitrogen-containing base, a pentose sugar and a phosphate group Ⓟ.

Nitrogen-containing base

There are four different nitrogen-containing bases found in DNA and four in RNA. In DNA the bases are: adenine, guanine, thymine and cytosine. In RNA thymine is replaced by a similar base called uracil. The bases are often referred to by their first letters: A, G, T, C and U.

Two of the bases, adenine and guanine, are related to the chemical purine and are referred to as purines. The other three bases, thymine, cytosine and uracil, are related to the chemical pyrimidine and are referred to as pyrimidines. Purine has two rings in its structure; pyrimidine has one ring.

Pentose sugar

You saw in Chapter 2 that sugars with five carbon atoms are called pentoses. Two pentoses are found in nucleic acids, ribose and deoxyribose.

A nucleic acid containing ribose is called a ribonucleic acid (RNA). One containing deoxyribose is called a deoxyribonucleic acid (DNA). As the name suggests, deoxyribose is almost the same as ribose except that it has one fewer oxygen atoms in its molecule.

Phosphate group

The phosphate group gives nucleic acids their acid nature.

Question

1 Look at the structure of the nucleotide in Figure 6.2. By identifying the sugar and the base with labels in each case, draw a nucleotide that could be found:
   - **a** only in DNA
   - **b** only in RNA.

The structure of ATP

Although ATP (adenosine triphosphate) is not part of DNA or RNA, you will look at its structure here because it is also a nucleotide.

The structure of ATP is shown in Figure 6.3. Its three components are adenine, ribose and phosphate. Adenine plus ribose forms a sugar–base called adenosine. Adenosine can be combined with one, two or three phosphate groups to give, in turn, adenosine monophosphate (AMP), adenosine diphosphate (ADP) or adenosine triphosphate (ATP).

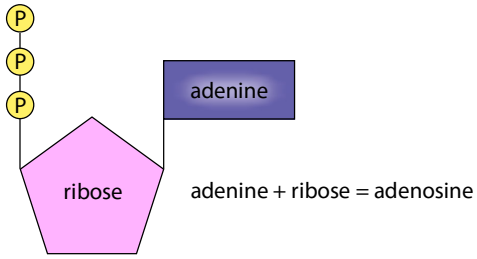


Figure 6.3: Structure of ATP, adenosine triphosphate.

Do not confuse adenine with adenosine, which is part of the name of ATP (adenosine triphosphate); adenosine is adenine with a sugar joined to it. And do not confuse thymine with thiamine; thiamine is a vitamin.

Dinucleotides and polynucleotides

Two nucleotides can be joined together by a condensation reaction (the same type of reaction used to join one amino acid to another, or one sugar to another).

Question

2 Name the bond formed when:
   - **a** two amino acids join by condensation
   - **b** two sugars join by condensation.

The molecule formed by joining two nucleotides is called a **dinucleotide** (Figure 6.4a). The bond formed is called a **phosphodiester bond**. The term *diester* is used because the phosphate group involved now has two ester bonds, one to each of the sugars it is connected to. The process can be repeated up to several million times to make a polynucleotide which has the form of a long, unbranching strand of nucleotides as shown in Figure 6.4b. The sugars and phosphates are linked by the phosphodiester bonds to form a backbone from which the bases stick out sideways at right angles to the backbone.

KEY WORDS

dinucleotide: two nucleotides joined together by a phosphodiester bond

phosphodiester bond: a bond joining two nucleotides together; there are two ester bonds, one from the shared phosphate group to each of the sugars either side of it

The structure of DNA

By the 1950s the strucure of polynucleotides as shown in Figure 6.4b was known, but this structure did not explain how DNA could store information or copy itself. A race was under way to solve this problem by learning more about the structure of DNA. The race was won in 1953 when James Watson and Francis Crick, working in Cambridge, England, published a model structure for DNA that turned out to be correct. According to James Watson, it was too pretty not to be true.

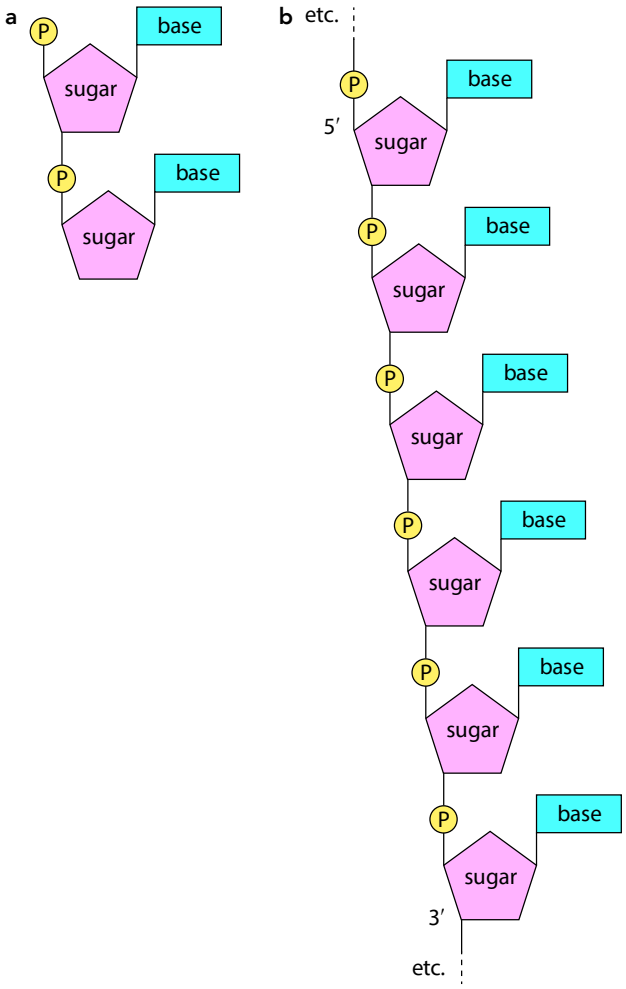


Figure 6.4: a Structure of a dinucleotide; **b** structure of a polynucleotide.

Question

3 The evidence used by Watson and Crick in building a model of DNA included data from an American chemist, Erwin Chargaff. Some of the relevant data are shown in Table 6.1. Can you spot a simple pattern in the base ratios?

The key to Watson and Crick's success was trying to make models of DNA using all the available evidence. Apart from Chargaff's data (see Question 3, Table 6.1), another vital piece of evidence came from X-ray diffraction photographs of DNA produced by Rosalind Franklin. Franklin worked at King's College in London (Figure 6.5) and her photographs suggested that DNA had a helical structure.

By playing with models, Watson and Crick eventually came up with the idea of the molecule having two polynucleotide chains, not one. The two chains could be held together by hydrogen bonding between the bases of the two chains. The model showed that the only way this could be done would be to twist each of the two strands into a helical shape – a double helix – and to run the strands in opposite directions. A sketch of this is shown in Figure 6.6.

Features of the DNA molecule

A model of DNA is shown in Figure 6.7a. A diagram of part of the molecule is shown in Figure 6.7b and Figure 6.8. The 5′ (say: five-prime) and 3′ (say: three-prime) on Figure 6.8 refer to carbon atoms 5 and 3 of the sugar. (See Chapter 2 for the numbering of carbon atoms in a sugar.) The two ends of a DNA strand are called the 5′ end and the 3′ end. At the 5′ end is phosphate and at the 3′ end is sugar.

Source of DNA	% Adenine	% Guanine	% Thymine	% Cytosine
human (mammal)	30.9	19.9	29.4	19.8
chicken (bird)	28.8	20.5	29.2	21.5
salmon (fish)	29.7	20.8	29.1	20.4
locust (insect)	29.3	20.5	29.3	20.7
wheat (plant)	27.3	22.7	27.1	22.8
E. coli (bacterium)	24.7	26.0	23.6	25.7
phage X174 (virus)	24.6	24.1	32.7	18.5

Table 6.1: Relative amounts of the four bases in different organisms. The numbers in each row add up to 100; each number is the percentage of that base in the DNA of the stated organism.

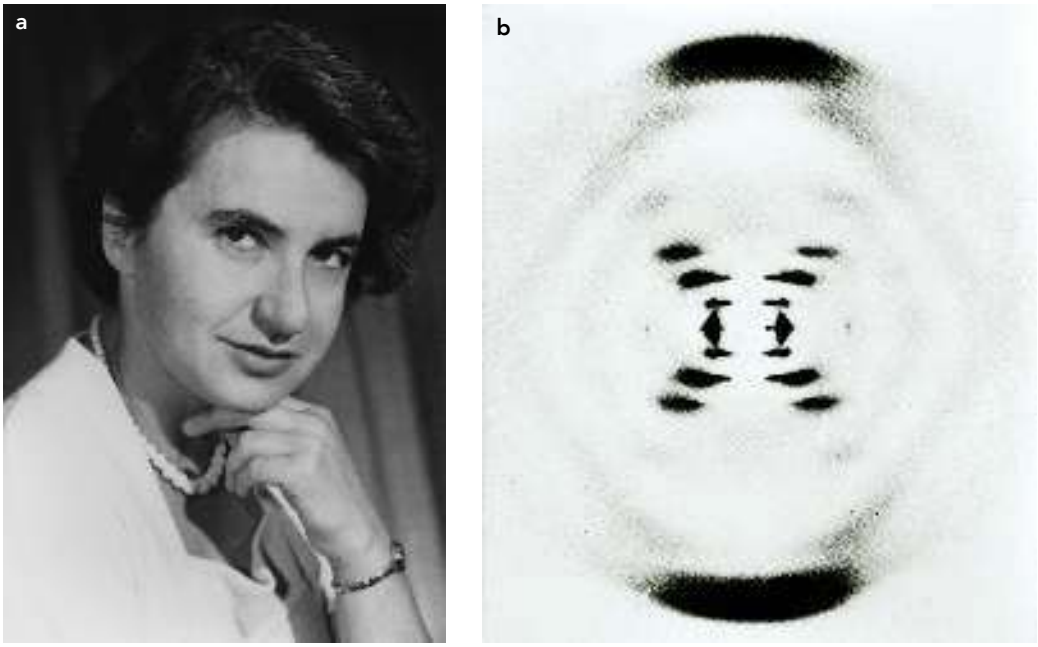


Figure 6.5: a Rosalind Franklin, whose X-ray diffraction images of DNA gave important clues to its structure; **b** X-ray diffraction photograph of a fibre of DNA.

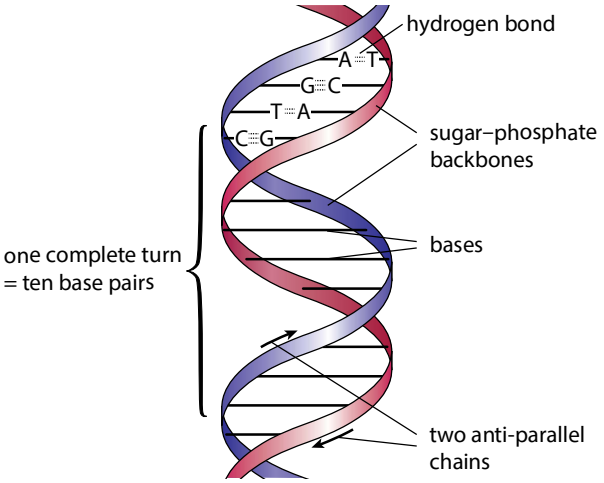


Figure 6.6: A sketch of the DNA double helix. The bases A, G, T and C are only shown in part of the molecule.

The DNA molecule has the following features:

- It is made of two polynucleotide chains.
- Each chain is a right-handed helix.
- The two chains coil around each other to form a double helix.
- The chains run in opposite directions – they are said to be antiparallel.
- Each chain has a sugar-phosphate backbone with bases projecting at right angles.
- The bases in one chain are attracted to the bases of the other chain by hydrogen bonding between the bases. This holds the chains together.
- Because of the way they fit together, like jigsaw pieces, adenine (A) always pairs with thymine (T) and guanine (G) always pairs with cytosine (C) (**complementary base pairing**). (This explains Chargaff's data – see Question 3.)

KEY WORD

complementary base pairing: the hydrogen bonding of A with T or U and of C with G in nucleic acids

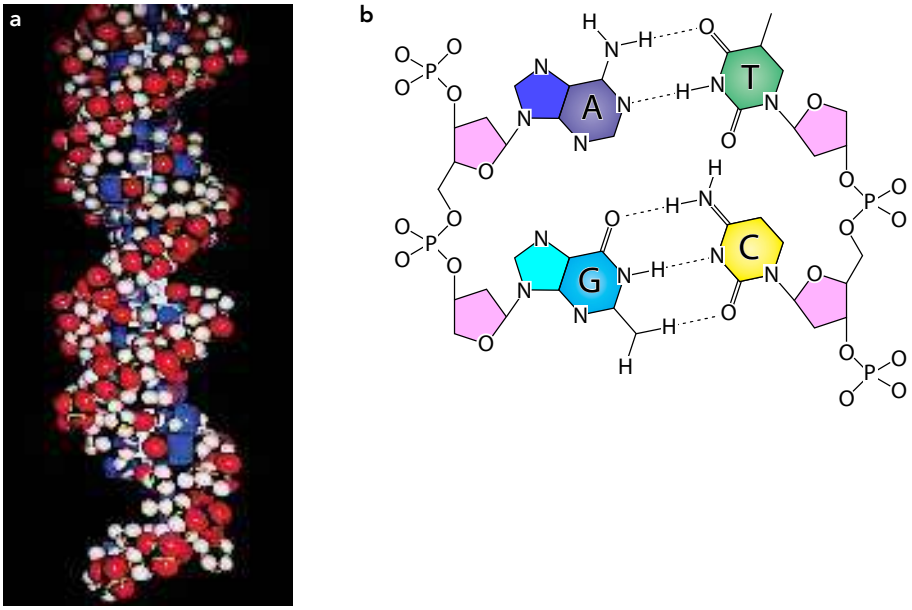


Figure 6.7: a A space-filling model of DNA. **b** A–T, G–C base pairs showing how neatly the bases fit together with hydrogen bonding. Note two hydrogen bonds between A and T, and three hydrogen bonds between G and C.

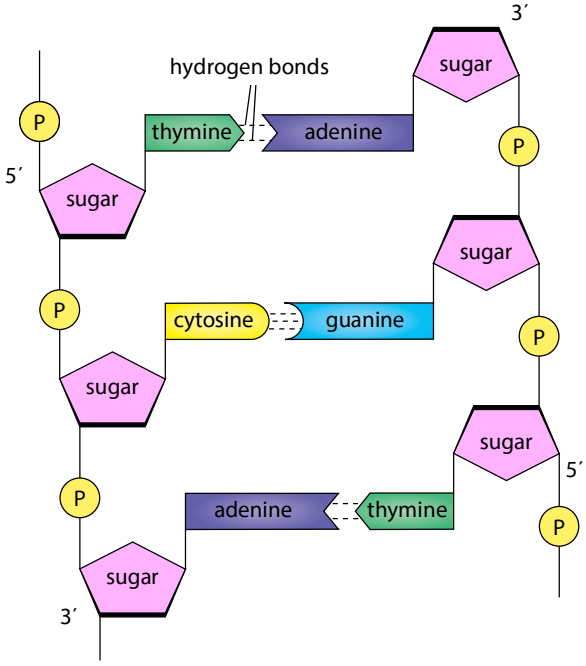


Figure 6.8: Part of a DNA molecule. Two polynucleotides, running in opposite directions, are held together by hydrogen bonds between the bases. A links with T by two hydrogen bonds; C links with G by three hydrogen bonds. This is complementary base pairing.

- A links with T by two hydrogen bonds; G links with C by three hydrogen bonds.
- Adenine and guanine are purines; thymine and cytosine are pyrimidines. A purine always pairs with a pyrimidine.
- Purines are two rings wide and pyrimidines are one ring wide; the distance between the two backbones is therefore constant and always three rings wide.
- A complete turn of the double helix takes place every 10 base pairs.

Because A must pair with T and G with C, the sequence of bases in one strand determines the sequence of bases in the other strand. The two strands are said to be complementary.

Watson and Crick realised immediately how this structure explained how DNA stores information and how it copies itself.

The information is the sequence of bases – represented by the four letters, A, G, T and C, in any order along the whole molecule. Any sequence is possible within one strand, but the other strand must be complementary. The *sequence* acts as a coded message.

The molecule can copy itself (replicate) accurately by 'unzipping' down the middle. This is relatively easy because the two strands are only held together by weak hydrogen bonds. Each half can then make a complementary copy of itself. Thus, two identical molecules of DNA are produced.

The structure of RNA

Unlike DNA, an RNA molecule is a single polynucleotide strand. Later in the chapter you will consider protein synthesis (Section 6.5). This involves three types of RNA, namely messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). Transfer RNA and rRNA fold up into complex structures, but mRNA remains as an unfolded strand.

6.3 DNA replication

Watson and Crick were quick to realise how DNA could replicate – copy itself. Scientists now know in detail how this process occurs and that it takes place during the S phase of the cell cycle (Chapter 5, Section 5.3, The cell cycle).

Replication is controlled by enzymes. It starts by the unwinding (separation) of the two strands of DNA by the breaking of the hydrogen bonds that normally hold the two strands together. This is the 'unzipping' mentioned earlier. The enzyme **DNA polymerase** is then used for the copying process. A molecule of DNA polymerase attaches to each of the single strands. It adds one new nucleotide at a time, which is held by hydrogen bonding to the strand being copied.

DNA polymerase can only copy in the 5′ to 3′ direction along each strand. This creates a problem. If you look at Figure 6.9, you can see that the top parent strand is being copied in the same direction as the unwinding process. The DNA polymerase simply follows the unwinding process, copying the DNA as it is unwound. The new strand being formed is called the **leading strand**. In contrast to the top parent strand, for the bottom parent strand the 5′ to 3′ direction of copying is in the opposite direction to the unwinding. This means that the DNA polymerase has to copy an unwound piece of DNA and then go back and copy the next piece of unwound DNA. It has to keep repeating this process. The result is a series of short fragments of copied DNA (Figure 6.9). These are called Okazaki fragments after the biochemist who discovered them. In this case, the new strand being formed is called the **lagging strand**.

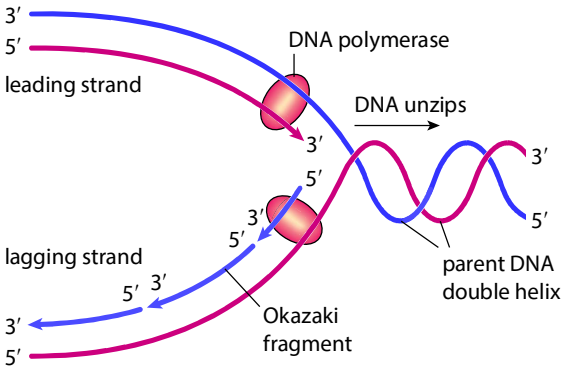


Figure 6.9: Diagram of DNA replication. The lagging strand can only be made in short fragments as the DNA unwinds.

Another enzyme called **DNA ligase** finishes the process. Its job is to connect all the new nucleotides with covalent bonds. Before this they are only holding on to the parent strand with hydrogen bonds between complementary bases. DNA ligase connects neighbouring nucleotides with phosphodiester bonds to form the sugar-phosphate backbone of the new DNA molecule. The Okazaki fragments are connected in the same way at the same time. Figure 6.10 shows you some more details of replication, including base pairing.

KEY WORDS

DNA polymerase: an enzyme that copies DNA; it runs along the separated DNA strands lining up one complementary nucleotide at a time ready for joining by DNA ligase

leading strand: during DNA replication, the parent strand that runs in the 3′ to 5′ direction is copied to produce the leading strand

lagging strand: during DNA replication, the parent strand that runs in the 5′ to 3′ direction is copied to produce the lagging strand

DNA ligase: an enzyme that catalyses the joining together of two nucleotides with covalent phosphodiester bonds during DNA replication

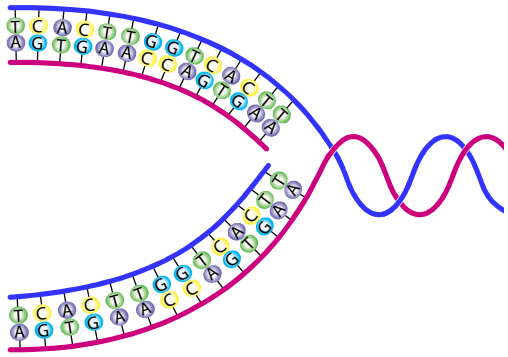


Figure 6.10: DNA replication showing base pairing.

Semi-conservative replication

The method of copying DNA described is called **semi-conservative replication**. This is because each time a DNA molecule is replicated, half the original molecule is kept (conserved) in each of the new molecules. If replication was conservative, the parent DNA molecule would remain at the end of the process and the new DNA molecule would have two newly made strands.

KEY WORDS

semi-conservative replication: the method by which a DNA molecule is copied to form two identical molecules, each containing one strand from the original molecule and one newly synthesised strand

gene: a length of DNA that codes for a particular polypeptide or protein

Questions

4 a Make a list of the different molecules needed for DNA replication to take place.
  b State what each of these molecules does.
  c In what part of a eukaryotic cell does DNA replication take place?

5 Figure 6.11 is a diagram of a DNA molecule. One strand is red and one is blue.
  Using the colours red and blue as appropriate, draw the two daughter molecules resulting from replication of this parent molecule:
  a if replication is semi-conservative
  b if replication is conservative.

Figure 6.11: DNA – what happens in replication?

6.4 The genetic code

As soon as the race to find the structure of DNA had been won, a new race started – a race to break the genetic code. Watson and Crick had realised that the DNA code was the sequence of bases in the DNA. Scientists also realised that the sequence of bases must be the code for the sequences of amino acids in proteins.

Here is the logic that led to this realisation. The activities of a cell are controlled by enzymes. Enzymes are proteins. Each type of protein has a unique sequence of amino acids which determines its structure and, hence, its function. Therefore, if you control the sequences of amino acids in the cell's proteins, you control the enzymes that are made and thus you control the cell. The sequence of bases in the DNA of a cell is the code for all the proteins of that cell and organism. The code for one polypeptide is called a **gene**.

There are 20 common amino acids found in proteins, but only four different bases in DNA to code for them. It cannot be the case that each base codes for one amino acid. Even if two bases were the code for one amino acid, there would be only 16 possible codes: AA, AG, AT, AC, GA, GG, GT, GC, TA, TG, TT, TC, CA, CG, CT, CC. This is still not enough codes for 20 amino acids.

If the code were a triplet code, there would be 64 possible combinations of three bases. Although this seemed too many to code for only 20 amino acids, it *is* the case because most of the amino acids have more than one code. The nature of the code was gradually broken and, by 1964, it was possible to look up any code in a table like Appendix 2.

Question

6 Can you work out the general formula for predicting how many amino acids can be coded for by four bases?