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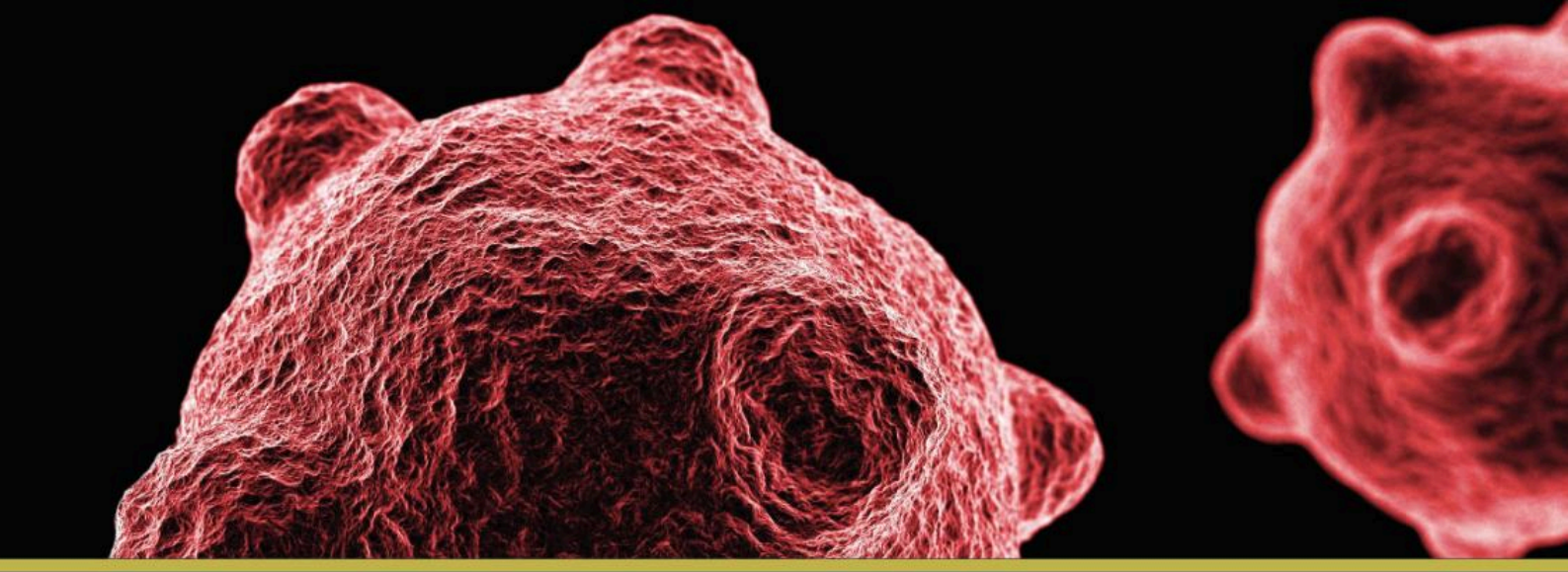
for Cambridge International AS & A Level

Richard Fosbery



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Contents

Introduction

The *Exam Success* series will help you to reach your highest potential and achieve the best possible grade. Unlike traditional revision guides, these new books give advice on improving answers, helping to show you what examiners expect of candidates. All the titles are written by authors who have a great deal of experience in preparing candidates for exam success.

Exam Success in Cambridge International AS and A Level Biology covers the requirements of the AS Level and A Level Cambridge 9700 Biology syllabus. The first 19 units cover the syllabus content, with exam-style questions at the end of each unit. Unit 20 provides advice on experimental skills for paper 3 and help with planning, analysing and evaluating experiments for paper 5. Unit 21 consists of questions in the styles of the five exam papers, with exam tips to support your work.

Throughout the book, a grey vertical bar shows AS content, and a pink bar shows A Level content; key terms are also highlighted.

Each *Exam Success* book has common features to help you do your best in the exam:

Key points

☐ These are based on the learning outcomes in the syllabus. Check them off when you can write confidently about each one.

Worked example

These give examples of questions, and show you how best to answer them.

Practical skills

These describe practical skills that you might be tested on, and are intended as reminders of work you may have already done in the lab.

Remember

These include key information that you must remember if you are to achieve a high grade.

Raise your grade

Here, you can read answers that achieved maximum marks, as well as find out how to improve answers where marks were lost. Full mark schemes are provided on the OUP support website.

Exam-style questions

These questions are at the end of each unit. Additional questions are on the OUP support website together with mark schemes.

Access your support website for additional content here:
www.oxfordsecondary.com/9780198409908

Key terms

These give easy-to-understand definitions of important terms.

Link

These show where in the book you can find more information about the topic.

A qualified teacher should carry out a risk assessment for each task before they are used as a class practical. Safety goggles and a laboratory coat must be worn where it is appropriate to do so. The author and the publisher cannot accept any responsibility for safety.

Exam tip

These provide advice on your revision and guidance to help you understand exactly what examiners are looking for.

Maths skills

These remind you of the vital mathematical skills that you need in order to answer exam questions in biology.

1 Cell structure

Key points

- ☐ Define resolution and magnification as applied to microscopy.
- ☐ State the approximate resolution of the light microscope (LM) and the transmission electron microscope (TEM).
- ☐ Recognise images as taken with the LM and with TEM and the scanning electron microscope (SEM).
- ☐ Do calculations to determine the linear magnification of images of cells and the actual sizes of cells and cell structures from photomicrographs, electron micrographs, drawings and diagrams.
- ☐ Compare the structure and function of prokaryotic cells and eukaryotic cells including plant cells and animal cells and their cell structures.
- ☐ State that ATP is produced in mitochondria and chloroplasts and outline the role of ATP in cells as an energy transfer molecule.
- ☐ State that viruses are non-cellular.

The cell

A cell is a basic unit of life composed of cytoplasm surrounded by a cell surface membrane.

There are two types of cellular organisation: **prokaryotes** and **eukaryotes**.

Viruses are non-cellular as they have no cell structure. They have genetic material (RNA or DNA) surrounded by a protein coat. Some viruses also contain a few enzyme molecules. Viruses typically range in size from 20 to 400 nm. Enveloped viruses (e.g. human immunodeficiency virus, HIV) are covered in a phospholipid and protein membrane formed from the cells of their hosts.

The linear dimensions of plant, animal and bacterial cells are usually stated in micrometres, µm (1/1000th of a mm). The dimensions of the usually much smaller viruses are often stated in nanometres, nm (1/1000th of a µm).

Microscopes are used to study the structure and function of cells. Living cells can be observed under the **light microscope**.

The cells of flowering plants and the cells of mammals are examples of eukaryotic cells. Plant cells are always surrounded by a cellulose cell wall, whereas animal cells do not have cell walls.

The cell surface membrane of eukaryotic cells encloses many different cell structures (sub-cellular structures). These include various distinct organelles and the cytoskeleton (the 'cell skeleton') (see Tables 1.1 and 1.2).

Most cell structures can only be seen with an **electron microscope**. You can see from electron micrographs that cells are divided into compartments. Many processes that occur in cells require different conditions, so they are localised into these compartments. For example, lysosomes are required only when a cell needs to break down 'worn out' cell structures and material it has taken in, or when a cell destroys itself. The membrane surrounding lysosomes protects the cell as lysosomes contain enzymes that digest

Key terms

Eukaryote: an organism that has cells with true nuclei and membrane-bound **organelles,** such as mitochondria.

Prokaryote: an organism that has cells without nuclei and membrane-bound organelles.

Link

There is more about HIV in Unit 10.

Find diagrams and electron micrographs of different types of virus so you get an idea of the variety of shapes and size.

Link

You can revise the structure and function of cell surface membranes in Unit 4.

biological molecules and they work best in a pH lower than in the rest of the cell.

Cells carry out various processes and activities to fulfil their specialised functions:

- obtain energy and convert it into usable form
- gain raw materials from their surroundings
- produce biological molecules, such as carbohydrates, lipids, proteins and nucleic acids
- package materials so they can be exported from the cell
- excrete waste materials
- store and use genetic information.

Cell structures work together to carry out these functions in each cell (Table 1.1). When a cell makes and secretes a certain protein, it is the nucleus, mitochondria, the rough endoplasmic reticulum (RER), Golgi body, Golgi vesicles, microtubules and the cell surface membrane that all work together. These cell structures are involved in the different stages between making copies of the gene that codes for the protein and the fusion of secretory vesicles to the cell surface membrane and the release of their contents.

Link

There is more about the function of lysosomes in Unit 11.

▼ **Table 1.1** The structures in eukaryotic cells, their features and functions

Cell structure	Feature	Function(s)
cell surface membrane	bilayer of phospholipid with proteins (details are in Unit 4)	cell boundary retains cell contents controls exchange of substances with surroundings
nuclear envelope	a double membrane, with ribosomes only on the outer membrane, which is continuous with RER nuclear pores	separates nucleus from cytoplasm allows movement of substances between the cytoplasm and the nucleus
nucleus*	clearly visible in LM and EM when stained	contains store of genetic information as DNA in chromosomes (see Unit 5)
nucleolus*	darkly staining area in nucleus	produces the large and small subunits of ribosomes
rough endoplasmic reticulum (RER)	flat sacs of membrane enclosing fluid-filled lumen (space) outer surface is covered in ribosomes	ribosomes carry out protein synthesis RER modifies proteins and transports them to the Golgi body
smooth endoplasmic reticulum (SER)	similar to RER but more tubular and with no ribosomes on the outer surface	makes triglycerides (fats), phospholipids, cholesterol
Golgi body (or Golgi apparatus)	pile of flat sacs with vesicles forming around the edge	modifies and packages proteins makes vesicles for secretion makes vesicles that become lysosomes
mitochondrion (plural: mitochondria)	formed of two membranes surrounding a fluid-filled matrix that contains 70S* (smaller) ribosomes and circular DNA inner membrane is highly folded to give large surface area for enzymes for respiration	site of ATP production by aerobic respiration (see Unit 12)

80S+ (larger) ribosome	attached to RER or free in cytoplasm – made of protein and RNA	assembles amino acids to make polypeptides
lysosome	single membrane that surrounds a fluid filled with enzymes	some enzymes destroy worn out parts of the cell and some digest food particles and bacteria
microtubules	very small, hollow tubes composed of molecules of the protein tubulin	form part of the cytoskeleton provide pathways in the cytoplasm for movement of structures, such as vesicles form the fibres that make up the spindle during nuclear division (see Unit 5)
centrioles (animal cells only)	located near to the nucleus in a non-dividing cell in cross section they have a 9 + 2 structure (9 outer pairs of microtubules in a ring with 2 in the centre)	may be involved in the assembly of microtubules to form spindle fibres (see Unit 5)
chloroplast* (plant cells only)	surrounded by two membranes with many internal membranes giving large surface area for chlorophyll, other pigments and enzymes	site of all the reactions of photosynthesis (see Unit 13)
cell wall (plant cells only)*	made of cellulose (cell walls of fungi are made of chitin)	resists the pressure of the contents of the cell to provide support
plasmodesma (plural: plasmodesmata) (plant cells only)	small 'tube' of cytoplasm, lined by the cell surface membrane, that connects adjoining cells (see Unit 7)	allows substances to move between cells without crossing cell surface membranes and cell walls
large permanent vacuole surrounded by tonoplast (plant cells only)*	filled with cell sap – a solution of ions and sugars, often with other substances such as pigments	creates a pressure that is exerted on the cell wall to provide support site for storing substances such as ions

*Structures easily visible in permanent and temporary preparations of cells and viewed with typical school/college LMs. A good LM can also be used to see mitochondria, Golgi bodies and the passages between the cell walls that indicate the position of plasmodesmata.
+S, Svedberg unit; used to measure small cellular structures and macromolecules. It refers to how the structures form sediment when they are spun in a centrifuge.

The cell structures can be divided into groups as shown in Table 1.2.

▼ **Table 1.2** The cell structures of eukaryotic cells that are surrounded by membranes and those that are not made of membranes

Cell structures surrounded by membranes		Cell structures not made of membranes
single membrane	**two membranes (double membrane)**	
Golgi body Golgi vesicles lysosome rough endoplasmic reticulum smooth endoplasmic reticulum vacuole of plant cells (surrounded by the tonoplast)	nucleus – surrounded by nuclear envelope chloroplast – surrounded by an envelope of two membranes mitochondrion – surrounded by outer and inner membrane; infoldings of inner membrane are cristae	nucleolus ribosomes cytoskeleton, composed of microtubules and other fibres centrioles (made of microtubules)

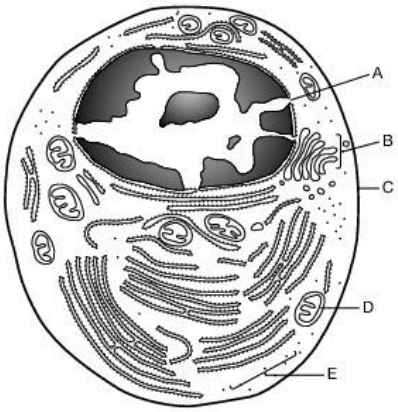
The protein-rich liquid that makes up the rest of the cytoplasm is called **cytosol**. Many reactions occur here, such as some of the reactions of respiration.

Worked example

Figure 1.1 is a drawing made from TEMs of mammalian cells.

The table shows some functions of the mammalian cell.

a) Complete the table by indicating the name of the structure that carries out each function and the appropriate label on Figure 1.1. One row has been completed for you.



▲ **Figure 1.1**

Function	Name of cell structure	Letter on Figure 1.1
aerobic respiration		
production of polypeptides		
allows movement between nucleus and cytoplasm		
packaging of proteins for export from the cell		
control of movement into and out of the cell	cell surface membrane	C

b) Many of the cell structures visible in Figure 1.1 are not visible in a light microscope. Explain why.

Answers

a)

Function	Name of cell structure	Letter on Figure 1.1
aerobic respiration	mitochondrion	D
production of polypeptides	rough endoplasmic reticulum	E
allows movement between nucleus and cytoplasm	nuclear pore	A
packaging of proteins for export from the cell	Golgi body	B
control of movement into and out of the cell	cell surface membrane	C

b) Structures such as ribosomes are very small; their size is below the resolution of the light microscope. The resolution is half the wavelength of light.

To help your revision, draw large diagrams of plant and animal cells as seen with the TEM on A3 or poster-sized paper. Label your diagrams and annotate the different structures with their functions.

Annotate means to add notes to your diagram. These can be brief or lengthy. There are annotated diagrams and drawings throughout this book.

Studying cells with microscopes

Practical skills

Resolution and magnification

The **resolution** of the human eye is about 0.2 mm (200 µm). If two structures are separated by a minimum distance of 0.2 mm, you can see them as two structures; otherwise they appear to you as one structure.

The resolution of the LM is 0.2 µm (200 nm), so anything smaller than this is not visible in the LM and to view them you need to use a device with a resolution *less than* 0.2 µm.

The resolution of TEMs used for many biological images is about 0.5 nm. This means membranes and ribosomes are visible. Anything smaller than 0.5 nm cannot be seen in many electron micrographs of cells.

Resolution determines the highest useful **magnification** that is possible in a microscope. If the resolution is poor it does not matter how much the image is magnified, no further detail is seen. The highest useful magnifications are:

- light microscope: ×1000 to ×1500
- transmission electron microscope: about ×250 000
- scanning electron microscope: ×100 000.

Key terms

Resolution: the ability to distinguish between two structures that are close together.

Magnification: the ratio between the actual size of an object and the size of an image, such as a drawing or a photograph.

Link

Membranes are about 7 nm thick and ribosomes vary in size with a diameter of ≤ 30 nm. The sizes of these structures are below the resolution of the LM, but they can be resolved in the EM.

Cells need energy

Adenosine triphosphate (ATP) is the energy transfer molecule in cells. Chloroplasts and mitochondria both produce ATP in cells. Some is also made in the cytosol.

ATP produced in chloroplasts is used to make sugars during photosynthesis and is not transferred to the rest of the cell. ATP is made in mitochondria during aerobic respiration. Some of the ATP produced in mitochondria is used within the organelle, for example for protein synthesis, but most is transferred to the rest of the cell for its energy needs. ATP does not travel from cell to cell.

Cells from mesophyll (photosynthetic) tissue and guard cells in leaves have many chloroplasts to maximise the photosynthesis that they can carry out. Cells with high energy demands, such as root hair cells, ciliated epithelial cells (see Unit 9) and plasma cells (see Unit 11) have many mitochondria.

Link

There is more about ATP in Unit 12 and Unit 13.

Exam tip

Search online for electron micrographs of liver cells as these have a high demand for ATP and so have many mitochondria. This will help you recognise different cell structures.

Eukaryotic cells have a more complex structure than prokaryotic cells

Table 1.3 compares the structure of prokaryotic and eukaryotic cells.

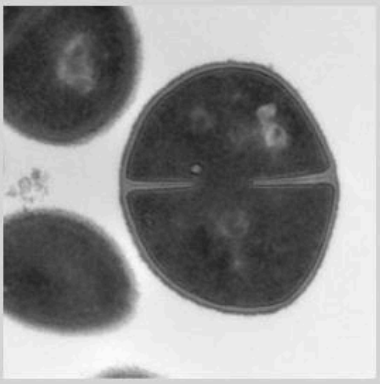
▼ **Table 1.3** The differences between prokaryotic cells and eukaryotic cells

Feature	Prokaryotic cell	Eukaryotic cells	
		Plant cells	Animal cells
typical size/µm	1.5 (range 0.5–3.0)	40–60	20
capsule/slime layer	found in some	✘	✘
cell wall	✔ (peptidoglycan, not cellulose)	✔ (made of cellulose)	✘

Feature	Prokaryotic cell	Eukaryotic cells	
		Plant cells	Animal cells
double membrane-bound structures	✘	✔	✔
single membrane-bound structures	✔ (storage vacuoles)	✔ (various, e.g. permanent vacuole)	✔ (various, e.g. lysosomes)
ribosomes	only small/70S	small/70S and large/80S	small/70S and large/80S
(true) nucleus	✘ (no nuclear envelope or nucleolus)	✔ (nuclear envelope and nucleolus present)	✔ (nuclear envelope and nucleolus present)
plasmids (small circular DNA)	✔	✘	✘
DNA	bacterial chromosome is made of a circular (closed loop) molecule of DNA in the cytoplasm	each chromosome is made of linear molecules of DNA in complexes with histone proteins found within the nuclear envelope	

Maths skills

Calculating magnifications and actual sizes



◀ **Figure 1.2** A transmission electron micrograph of a section through the bacterium *Staphylococcus aureus*. This cell is dividing by binary fission

Link

Some forms of *S. aureus* are causative organisms of infectious (communicable) diseases. You can revise information on the bacteria that cause cholera and tuberculosis in Unit 10.

Scale bars are one way to indicate the real size of the object that is very much smaller than the image. You can use the scale bar in Figure 1.2 to estimate the diameter of the bacterial cell, either by eye or by placing a ruler across the image. To make an accurate measurement, use a ruler to measure the length of the scale bar in millimetres and a linear measurement on the image (e.g. the diameter of the cell in Figure 1.2 at the widest place).

Use this formula to calculate the actual size:

$$\text{actual size} = \frac{\text{distance on image}}{\text{length of scale bar}} \times \text{length given on the scale bar}$$

Exam tip

Look at the unit on the scale bar and make sure that you change your measurement to the same unit. For example, if you have measured in millimetres, multiply by 1000 to give the measurement in micrometres.

Worked example

Use the formula to calculate the actual diameter of the cell of *S. aureus* in Figure 1.2.

Distance across cell at widest part = 35 mm = 35 000 µm

$$\text{actual size} = \frac{35\,000\ \mu\text{m}}{7\,000\ \mu\text{m}} \times 0.2\ \mu\text{m}$$

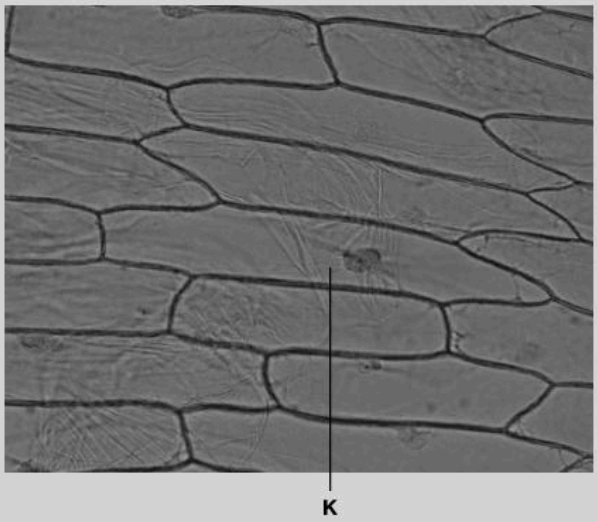
$$= 1.0\ \mu\text{m}$$

Note that the length of the scale bar in millimetres is multiplied by 1000 to give the measurement in the same unit (micrometre) as that printed on the scale bar.

Images may not have scale bars. Instead the magnification is given. Figure 1.3 shows some plant cells.

Use the magnification from the caption to calculate the actual length of one of the cells:

$$\text{actual length} = \frac{\text{length of image}}{\text{magnification}}$$



▲ **Figure 1.3** A photomicrograph showing epidermal cells from a scale leaf of an onion bulb (×280)

Link

Measurements like this should be given with the degree of uncertainty involved. You can find out what this means and how to determine uncertainty in Unit 20 Practical assessment.

Worked example

To measure the length of cell **K**, place a ruler to measure the full length of the cell. This should give a measurement of 61 mm. Use the formula:

$$\text{actual length} = \frac{61}{280}\,\text{mm}$$

$$\text{actual length} = 0.22\,\text{mm} = 220\,\mu\text{m}$$

Exam tip

Always measure in millimetres.

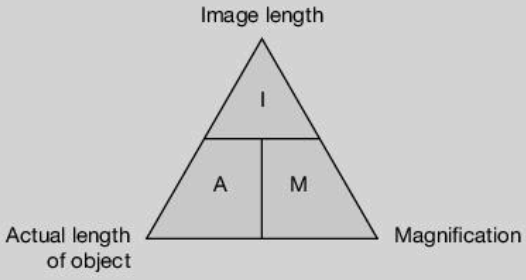
Exam tip

In a calculation question, always check what you are asked to do. You may be asked to give your answer to the nearest whole number and to use certain units. If you are not told which units to use, choose the most appropriate that give answers within the number range 1 to 100.

To calculate the magnification of an image, you need to divide the length of the image by the actual length of the object:

$$\text{magnification} = \frac{\text{length of the image}}{\text{actual length of the object}}$$

Use the triangle in Figure 1.4 to manipulate the formula for magnification.



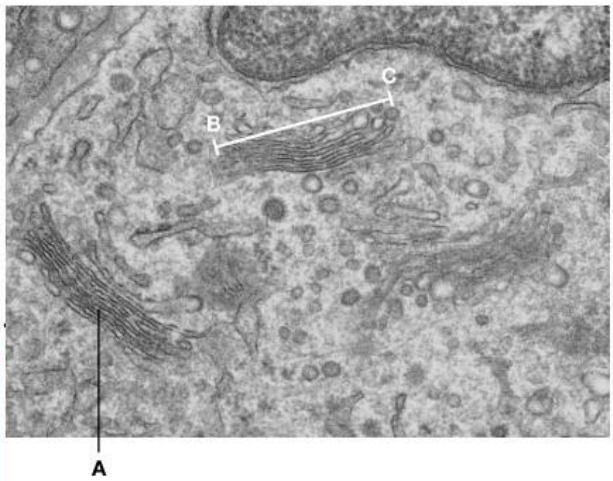
▲ **Figure 1.4** The 'magnification triangle'. Look at the formula for calculating magnification and the formula for calculating actual size to see how the triangle can help you remember which one to use

Exam tip

Make sure that you use the same units for the numerator (above the line in the calculation) and the denominator (below the line).

Raise your grade

1 The image shows a section through part of a secretory cell from the mammalian pancreas. The cell secretes digestive enzymes into the pancreatic duct to pass to the small intestine.



(a) (i) Name the cell structure labelled **A**. [1 mark]

Golgi ✔

Mark is awarded as many people call this structure 'the Golgi'. However, if asked to name a structure, give the full name as listed in the syllabus (Golgi body or Golgi apparatus or Golgi complex).

(ii) The magnification of the image is ×20 000.

Calculate the actual length of the structure as shown by the line **B–C**:

- write out the formula that you will use
- use the formula to calculate the actual length
- give your answer to the nearest 0.1 µm. [3 marks]

formula actual length = $\frac{\text{actual length on photo}}{\text{magnification}}$ ✔

Correct formula.

length of B - C = 2.4 cm, converted into µm = 2.4 × 1000 = 2400 ✘

The line B–C is in centimetres not in millimetres. There are 10000 µm in a centimetre so conversion is incorrect by a factor of 10.

$\frac{2400}{20\,000} = 0.12$ µm ✘

The answer is not rounded up / down to the nearest 0.1 µm as stated in the question. The correct answer = 1.2 µm.

(b) Explain why membranes are not visible in the light microscope, but are visible in electron micrographs like the one shown earlier. [3 marks]

The width of cell membranes is about 7 nm. ✔ This is too small to be seen in a light microscope.

A full explanation of this is needed that refers to resolution – resolution of an LM is 0.2 µm or 200 nm, which is nearly 30 times greater than the width of membranes.

(c) Suggest and explain the role of structure **A** in pancreatic cells. [3 marks]

The Golgi body processes proteins and puts them into vesicles ✔ so they can be moved around the cell.

One correct suggestion of role.

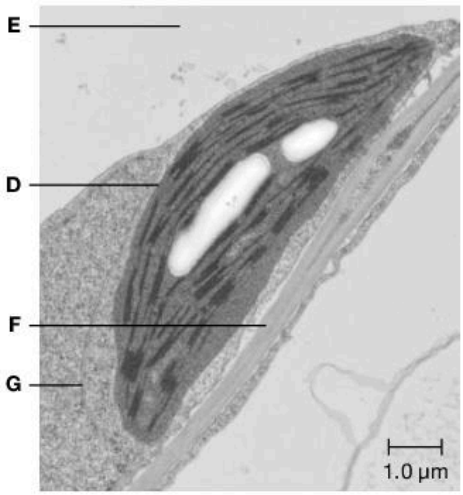
Too vague – could give an example of the processing by explaining that sugar molecules are added to proteins to make glycoproteins or, for the role of this organelle, use the information at the start of the question to say that digestive enzymes are packaged for release at the cell surface membrane by exocytosis.

2 (a) Viruses are described as non-cellular. Explain how the structure of a virus differs from the structure of a prokaryotic cell. [3 marks]

Viruses have no cell wall ✔ and no cytoplasm. ✔

Correct answers as prokaryotic cells have cell walls and cytoplasm. It is a good idea to give a full comparison and give three structural features not just two.

(b) The electron micrograph shows a small part of a cell from a young shoot of thale cress, *Arabidopsis thaliana*.



(i) Name the structure labelled **D** and state its function. [2 marks]

D is a chloroplast ✔ and it carries out photosynthesis. ✔

Both answers are correct.

(ii) Explain the role of the vacuole (**E**) and the cell wall (**F**) in giving support to plant cells. [2 marks]

The vacuole is full of water and has a hydrostatic pressure that pushes outwards onto the cell wall. ✔ The cell wall is made of cellulose and cannot stretch very much so it stops the vacuole expanding. The cell is turgid. ✔ If all the cells in plant organs (leaf, stem) are like this then the plant is supported and does not wilt.

Both answers are correct.

(iii) The structure labelled **G** is the nuclear envelope. State the evidence visible in the electron micrograph for this identification. [1 mark]

The nuclear envelope is made of two membranes with a small gap between them. ✔ In the EM you can see this clearly.

Correct answer.

(iv) Explain how you would determine the magnification of the image of the electron micrograph. [2 marks]

The scale bar is 7 mm long and represents 1.0 µm. ✔ This means that the magnification is ×7000. ✘

The question '*how* to calculate the magnification' is not answered. The first step in the answer, stating the length of the scale bar, is correct.

The answer to **(iv)** shows why it is important to re-read the question after you have written your answer. Make sure that you have answered the question set. Candidates often read too fast and misinterpret questions and so lose marks that they should get.

Exam-style questions

1 Which pair of organelles are surrounded by a double membrane?

A centriole and ribosome

B chloroplast and nucleus

C Golgi body and mitochondrion

D lysosome and rough endoplasmic reticulum

[1]

2 Which of the following organelles does **not** contain DNA?

A chloroplast

B mitochondrion

C nucleus

D ribosome [1]

3 Plasmodesmata connect adjoining plant cells.

What is the function of plasmodesmata?

A allow substances to move between cells without crossing membranes

B hold the cell walls of adjoining cells together

C allow substances to move through cell surface membranes

D allow the formation of cell walls during cell division [1]

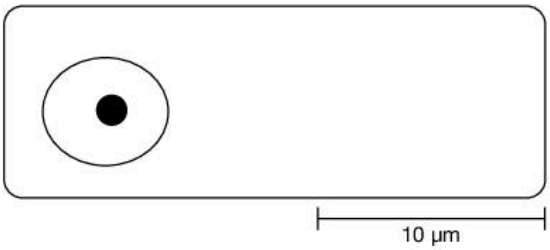
4 Cells can have 70S and 80S ribosomes and circular and linear forms of DNA.

Which letter in the table below represents a prokaryote?

	70S	80S	circular DNA	linear DNA
A	✔	✔	✔	✔
B	✔	✔	✔	
C	✔		✔	
D		✔		✔

[1]

5 The drawing shows an animal cell.



What is the diameter of the nucleolus?

A 0.45 nm

B 4.5 µm

C 1.3 µm

D 1.3 mm [1]

6 The following are structures found in cells.

1 Golgi body

2 lysosomes

3 nucleus

4 rough endoplasmic reticulum

5 smooth endoplasmic reticulum

Which structures are involved in the production of proteins in cells?

A 1, 3 and 4; B 1, 3 and 5;

C 2 and 4; D 2, 4 and 5 [1]

7 (a) Draw and label a diagram of a mitochondrion that is 50 mm long. [5 marks]

(b) Assume that the actual length of the mitochondrion is 3.5 µm, calculate the magnification of your drawing. [1 mark]

(c) State the function of the mitochondrion and explain how this benefits the rest of the cell. [2 marks]

(d) Explain why

(i) some cells have large numbers of mitochondria [2 marks]

(ii) fertilised eggs without any functional mitochondria do not survive. [2 marks]

extra questions available online

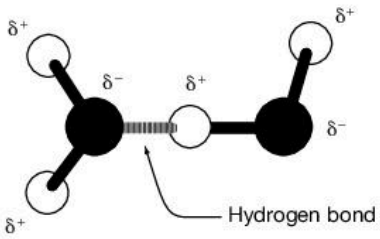
2 Biological molecules

Key points

- ☐ Explain how hydrogen bonding occurs between water molecules.
- ☐ Relate the properties of water to its role in living organisms.
- ☐ Define the terms monomer, polymer, macromolecule, monosaccharide, disaccharide and polysaccharide.
- ☐ Describe the ring forms of α-glucose and β-glucose.
- ☐ Describe the formation of glycosidic bonds by condensation and their breakage by hydrolysis.
- ☐ Describe the molecular structure of starch (amylose and amylopectin), glycogen and cellulose and relate their structures to their functions.
- ☐ Describe the formation of an ester bond between glycerol and a fatty acid.
- ☐ Describe the molecular structure of triglycerides and phospholipids and relate their structures to their functions.
- ☐ Describe the structure of an amino acid and the formation and breakage of peptide bonds.
- ☐ Explain the four levels of organisation in proteins and describe the bonds that hold protein molecules in shape.
- ☐ Describe the molecular structure of haemoglobin and collagen and explain how their structures are related to their functions.
- ☐ Describe and carry out chemical tests for starch, reducing sugars, non-reducing sugars, lipids and proteins.
- ☐ Describe and carry out a semi-quantitative test for a reducing sugar.

Water

There are **hydrogen bonds** between water molecules. The oxygen atom of water is slightly electronegative and the hydrogen atom is slightly electropositive so a water molecule is **dipolar**. The partial charges are indicated by δ (delta): δ^- on the oxygen and δ^+ hydrogen. A hydrogen bond forms between the slightly negative charge and the slightly positive charge (Figure 2.1). Each hydrogen bond is very weak and easily broken. Each water molecule can form hydrogen bonds with up to four other water molecules. In bodies of water, hydrogen bonds break and reform all the time.



▲ **Figure 2.1** Two water molecules with a hydrogen bond between them

As water molecules are polar they interact and are a good solvent for other polar molecules and ions. The hydrogen bonds between water molecules give them **cohesion** resulting in properties that are important within organisms and cells (Table 2.1).

▼ **Table 2.1** The properties of water related to its role within organisms

Property	Explanation	Roles of water within organisms
solvent for polar and charged substances which dissolve readily in water uncharged substances also dissolve, but less readily	polar molecules (e.g. glucose) and ions (e.g. Na^+, Cl^-) are attracted to the weak charges on water molecules	solvent within cells solvent in transport media, e.g. blood plasma; lymph; phloem sap and xylem sap solvent for excretory wastes in urine

Remember

An ion is an atom or molecule or chemical group that has lost or gained one or more electrons and is either positively or negatively charged.

AS Level

Property	Explanation	Roles of water within organisms
high specific heat capacity	4.2 Joules of energy are needed to increase the temperature of 1 g of water by 1 °C; this thermal energy breaks hydrogen bonds between water molecules	this property limits the fluctuations in the temperature of organisms
high latent heat of vapourisation	much thermal energy is needed to cause water to change to water vapour	loss of water for cooling (sweating in animals and in evaporation from mesophyll cells during transpiration in plants)

Carbon-based molecules

The complex compounds of carbon are known as **organic compounds**. All the major compounds that make up organisms are based on carbon, which forms very strong **covalent bonds** with itself and with other atoms. Carbon forms single and double covalent bonds.

The assembly of **macromolecules** from smaller molecules is part of anabolism (Table 2.2). Smaller molecules of the same type are **monomers**. Larger molecules made from many monomers are **polymers**. Lipids are *not* polymers as they are not made of repeated smaller molecules.

Remember

Compounds are substances made from two or more chemical elements. Organic compounds are all based on the element carbon.

Carbohydrates

Carbohydrates are organic compounds that:

- have the general formula $C_x(H_2O)_y$
- include the **monosaccharides** (e.g. glucose), **disaccharides** (e.g. sucrose) and **polysaccharides** (e.g. starch, glycogen and cellulose).

Key terms

Monosaccharide: the simplest type of carbohydrate with the formula $C(H_2O)n$; sugars with three to seven carbon atoms. The most commonly occurring monosaccharides are trioses (3C) (Units 12 and 13), pentoses (5C) (Unit 6) and hexoses (6C).

Disaccharide: a sugar molecule formed by joining two monosaccharides using a glycosidic bond; e.g. sucrose.

Polysaccharide: complex carbohydrate formed by joining many monosaccharides using glycosidic bonds to form branched molecules, e.g. glycogen, or unbranched molecules, e.g. amylose.

Link

Hydrogen bonds are important in the structure of cellulose (page 15), DNA (Unit 6 page 64) and proteins (page 18).

Exam tip

You might be asked to discuss how the structure and properties of water explain the roles of water in living organisms. Remember that hydrogen bonding is the key to the explanations.

Link

The high cohesion of water molecules supports columns of water in xylem (see Unit 7 page 79).

Remember

In a covalent bond a pair of electrons is shared between the atoms in a molecule. It is much stronger than an ionic bond.

Key terms

Macromolecule: any large molecule composed of smaller sub-unit molecules.

Monomer: any small molecule of the same type that is used to make larger molecules.

Polymer: any large molecule made of repeated monomer molecules, which may be identical (as in starch) or of the same type (as in proteins).

▼ **Table 2.2** Biological macromolecules

Macromolecules	Elements	Sub-unit molecules	Examples	Roles in organisms
Polysaccharides	C, H, O	α-glucose β-glucose	• starch (amylose and amylopectin) and glycogen • cellulose	• energy storage • structural–support
Lipids	C, H, O	glycerol, fatty acids phosphate (phospholipids)	• triglycerides • phospholipids	• energy storage, thermal insulation • structural–membranes and electrical insulation
Proteins	C, H, O, N, S	amino acids (20 different types)	• amylase, pepsin • insulin, glucagon • antibodies • haemoglobin • collagen	• catalysts • hormones • defence • transport • structural–support
Nucleic acids	C, H, O, N, P	nucleotides (five different types)	• DNA • RNA (mRNA, tRNA, rRNA)	• storage of genetic information • production of proteins

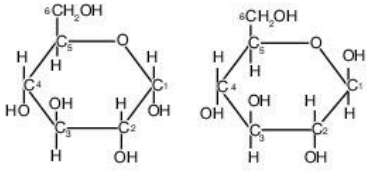
Monosaccharides

Glucose molecules – formula $C_6H_{12}O_6$ – have two forms: straight chains and rings.

In solution, most glucose is in one of two ring forms (Figure 2.2).

- α (alpha) has –H above the ring at position 1
- β (beta) has the –H below the ring at position 1.

These two forms of glucose are polymerised to form macromolecules with very different properties and roles in cells.



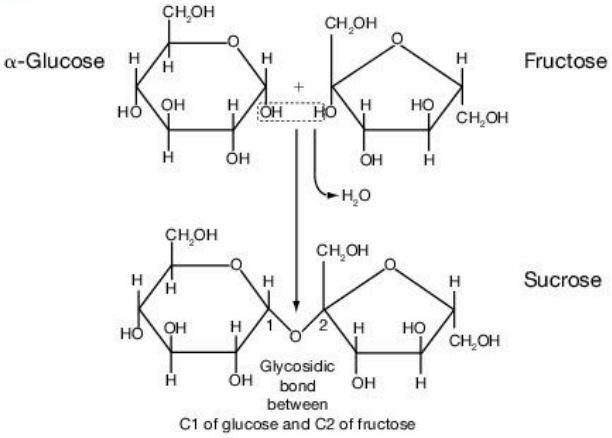
α-glucose β-glucose

▲ **Figure 2.2** α-glucose and β-glucose. The numbers refer to the carbon atoms. They are also referred to as C1, C2, etc.

All monosaccharides are **reducing sugars** because they have functional groups that can donate electrons when heated: the aldehyde group on C1 of glucose and the ketone group on C4 of fructose.

Formation of a disaccharide

In plant cells, the disaccharide sucrose is made from glucose and fructose. A molecule of water is removed so that an oxygen 'bridge' forms between C1 of the glucose molecule and C2 of the fructose molecule. A strong covalent **glycosidic bond** is formed. The reaction is a **condensation reaction**.



▲ **Figure 2.3** The formation of a molecule of sucrose by a condensation reaction

Key terms

Glycosidic bond: type of covalent bond that forms between monosaccharides by condensation. Also formed when a monosaccharide is added to a growing polysaccharide.

Condensation reaction: a type of anabolic reaction where the removal of water allows a bond to form.

Sucrose, which is polar and water soluble, is formed by plants for transport in the phloem. It is not as reactive as glucose and fructose because the aldehyde and ketone groups that form the glycosidic bond are not available to react with other molecules. This lack of reactivity makes sucrose a **non-reducing sugar** (see page 20).

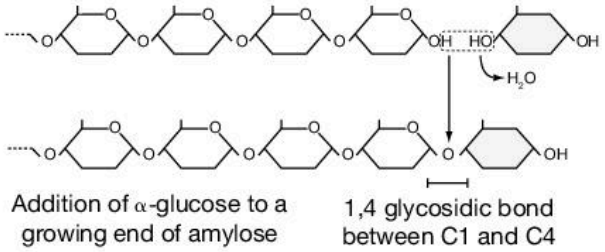
Metabolism is all the chemical reactions that occur in organisms divided into:

anabolism – reactions that build up larger biological molecules from small ones

catabolism – reactions that break down larger molecules to small ones.

Polysaccharides

Cells use glucose and other monosaccharides as monomers to make complex polysaccharides.



▲ **Figure 2.4** The formation of a glycosidic bond between the end of a polysaccharide and an α-glucose monomer

A glycosidic bond that forms between C1 at the end of the growing chain and C4 of the glucose monomer that is being added is a 1,4 glycosidic bond. Glucose monomers added like this form unbranched chains. A glycosidic bond formed by adding a glucose monomer to C6 on a growing chain is a 1,6 glycosidic bond, which forms a branching point. Another chain can then form with more 1,4 glycosidic bonds joining glucose monomers together (Figure 2.5).

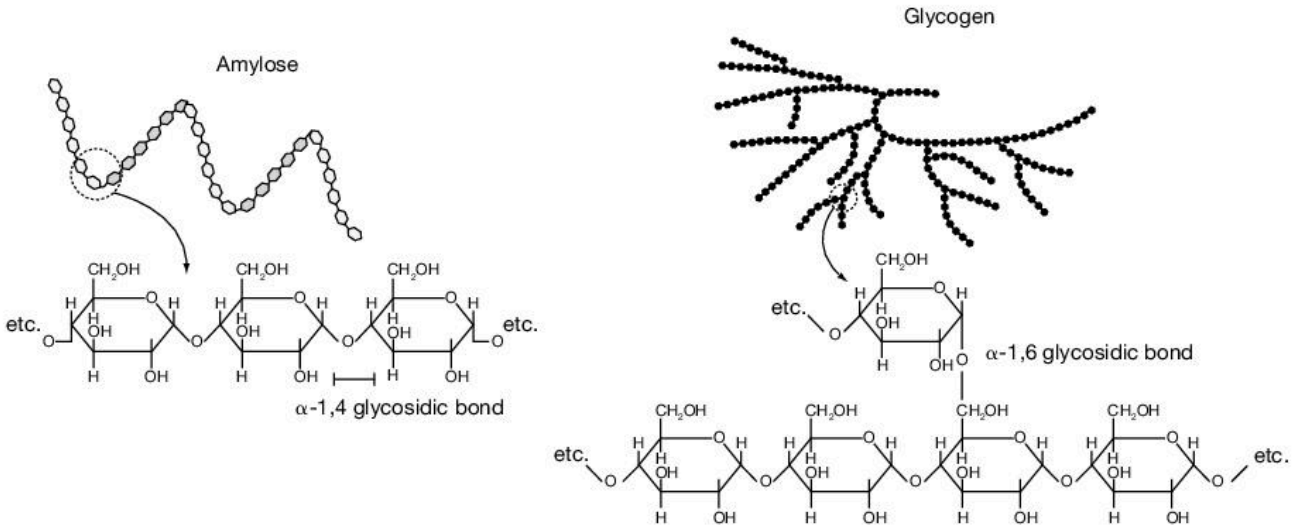
When broken down, glucose molecules are removed from the end of each chain by **hydrolysis**.

Three energy storage polysaccharides are made from α-glucose monomers:

- **amylose**
- **amylopectin**
- **glycogen**.

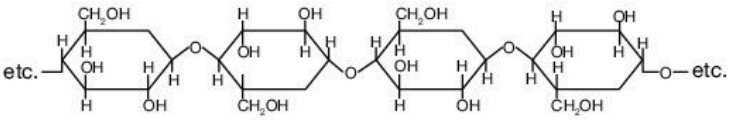
Once part of a larger molecule, monomers are often called residues because of the removal of one or more atoms when forming the bond between molecules.

Starch is composed of amylose and amylopectin. Glycogen is similar to amylopectin, but it has more 1,6 glycosidic bonds and therefore has many more branches.

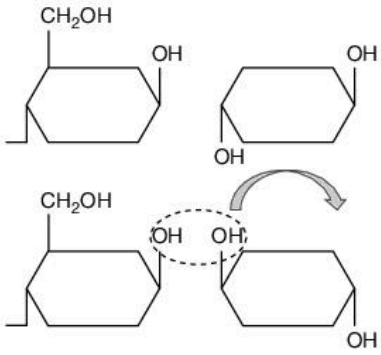


▲ **Figure 2.5** Two polysaccharides: amylose and glycogen

Cellulose is a long-chain molecule made from β-glucose monomers. Alternate β-glucose monomers are arranged at 180° to each other as they are added to a growing cellulose molecule. This is because the –OH group on C1 must be in the same orientation as the –OH group on C4 of the last glucose monomer in the chain (Figure 2.6). This alternating arrangement gives a straight chain, not a helix (Figure 2.7).



▲ **Figure 2.7** A small part of a molecule of cellulose showing the many –OH group projections on both sides of the chain



▲ **Figure 2.6** Forming glycosidic bonds between β-glucose monomers involves switching the orientation of alternate monomers

▼ **Table 2.3** The structure and roles of polysaccharides

Polysaccharide	Monomer	Glycosidic bonds	Structure	Role
starch amylose	α-glucose (200–5000)	α-1,4	• unbranched chain – right-handed helix	• energy storage in plants
amylopectin	α-glucose (5000–10000)	α-1,4 and α-1,6	• branched chain – not a helix	
glycogen	α-glucose (up to 120000)	α-1,4 and α-1,6	• branched chain, more branched than amylopectin	• energy storage in animals, fungi and some bacteria
cellulose	β-glucose (300–10000)	β-1,4	• unbranched • straight chain	• provides strength to cell walls of plants

Structure and function of carbohydrates

Energy storage

Amylose and amylopectin and glycogen are ideal for storage as they are compact and insoluble in water, so they don't affect the osmotic properties of the cell. The branches in amylopectin and glycogen have many 'ends' where glucose can be added or removed as required by a cell.

Support

Bundles of cellulose molecules bonded together by hydrogen bonds form very strong **microfibrils** that are then bound together to form cellulose fibres. The fibres are arranged in plant cell walls in a criss-cross pattern to provide additional strength. Each microfibril forms hydrogen bonds with other polysaccharides that form a matrix in between the microfibrils. These arrangements give a strong structure that can support plant cells by withstanding the high turgor pressures of cell contents caused by the absorption of water by osmosis (see Unit 4).

Lipids

Triglycerides and **phospholipids** are composed of glycerol and three attached sub-unit molecules. The carboxylic acid group of fatty acid sub-units reacts with an –OH group of glycerol to form an **ester bond**.

As lipids have many –CH groups rather than –OH groups, they are not polar and are insoluble in water.

Key terms

Triglyceride: a lipid composed of glycerol and three fatty acids.

Phospholipid: a lipid composed of glycerol, two fatty acids and a water-soluble phosphate group. Attached to the phosphate are other water soluble groups.

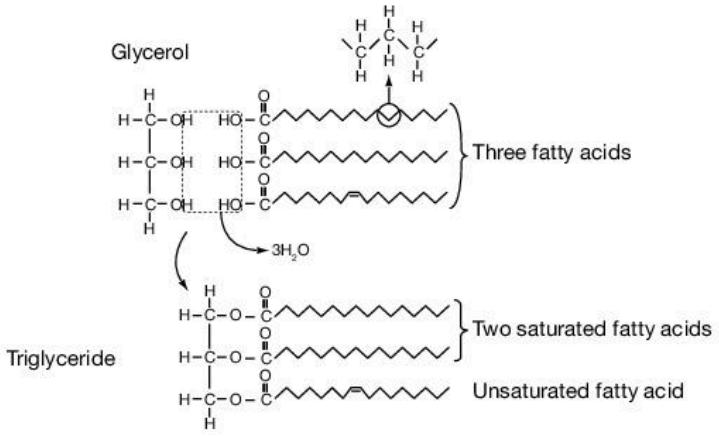
Ester bond: a type of covalent bond formed between an alcohol and an organic acid.

Triglycerides

There are different types of fatty acids:

- **saturated fatty acids** with a full complement of hydrogens attached to the carbon chain and no double bonds between carbon atoms in the chain
- **unsaturated fatty acids** with at least one double bond between carbon atoms along the carbon chain so there are fewer hydrogens.

Triglycerides can have three identical fatty acids or a mixture of different fatty acids including both saturated and unsaturated (Figure 2.8). Fatty acids have different numbers of carbon atoms in the hydrocarbon chain, usually even numbers between 12 and 22.



▲ **Figure 2.8** Glycerol and three fatty acids combine by condensation reactions to form a triglyceride

Link

Cholesterol and the steroid hormones (testosterone, oestrogen and progesterone) are other examples of lipids (see Units 4 and 15).

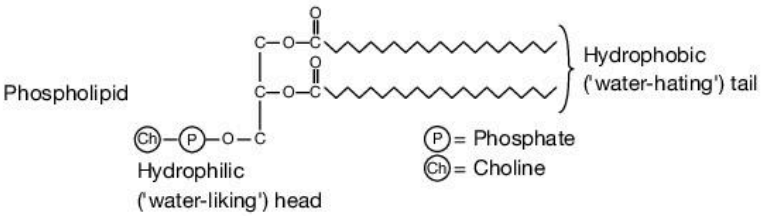
Function of triglycerides

Triglycerides make excellent long-term energy storage molecules. They are more efficient for energy storage than polysaccharides as they are highly reduced molecules because of the presence of many hydrogen atoms and far fewer oxygen atoms. When oxidised during respiration, much more energy is released than from the same mass of carbohydrate or protein.

Link

For more information about the energy content of carbohydrates, proteins and fats, see Unit 12.

Phospholipids



▲ **Figure 2.9** A phospholipid is composed of glycerol, two fatty acids and a phosphate-containing part

Fats and oils have the same chemical structure, but oils have a lower melting point than fats so are liquids at temperatures in the range 4–20 °C. Oils have a high proportion of unsaturated fatty acids.

Attached to the phosphate group is a water-soluble group, such as choline (Figure 2.9). This phosphate 'head' is water soluble, whereas the two fatty acids are not water soluble. When in contact with water, a single layer of phospholipids will form either a layer on top of the water or tiny spheres with the **hydrophilic** phosphate heads attracted to water and the **hydrophobic** fatty acid chains (sometimes called 'tails') in the centre.

Function of phospholipids

The cell surface membranes that surround cells are mainly composed of phospholipids. Two layers of phospholipids form a **phospholipid bilayer** with a central hydrophobic region and two hydrophilic regions in contact with water. The hydrophobic core of the membrane forms a barrier for many substances.

Link

All cell membranes have a phospholipid bilayer. This determines many of their properties (see Unit 4).

Proteins

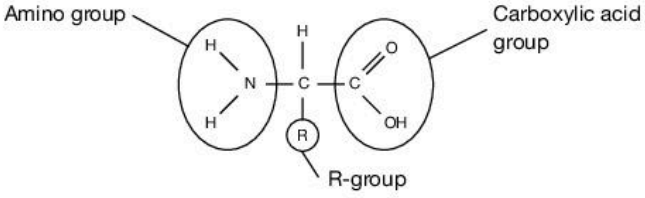
Proteins are macromolecules made from one or more chains of **amino acids** known as **polypeptides**. Polypeptides are unbranched molecules composed of approximately ten or more amino acids linked by **peptide bonds**.

Key term

Peptide bond: a covalent bond that forms between the carboxylic acid group of one amino acid and the amino group of another. The bond forms between a carbon atom and a nitrogen atom.

Amino acids

All **amino acids** share the same molecular structure shown in Figure 2.10.



▲ **Figure 2.10** A generalised amino acid molecule. Each type of amino acid has a different R-group, e.g. the R-group of glycine is –H

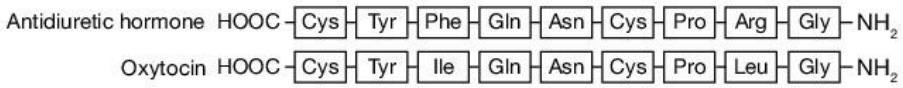
Exam tip

You could be given information about amino acid sequences in an exam paper. You are not expected to know any examples.

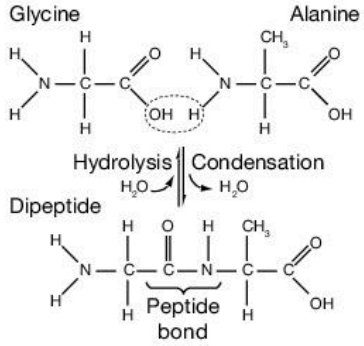
Cells use 20 different types of amino acid to make proteins.

Amino acids are joined by condensation reactions to form peptide bonds that link together the C of the carboxyl group of one amino acid with the N of the amino group of the other:

- dipeptides are formed from two amino acids
- tripeptides are formed from three amino acids
- oligopeptides ('oligo' means few) are formed from < 10 amino acids (see Figure 2.12)
- polypeptides consist of ⩾ 10 amino acids with a central chain of carbon and nitrogen atoms.



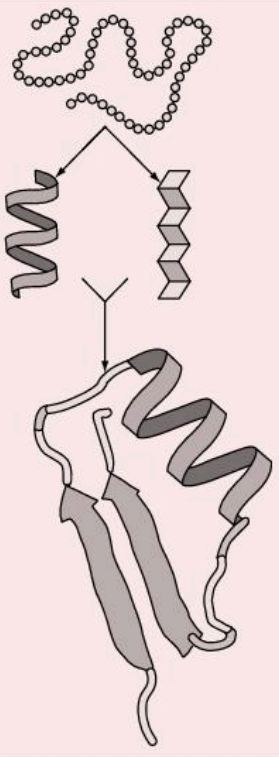
▲ **Figure 2.12** Two oligopeptides (with nine amino acids) that are biologically active: $-NH_2$ is the N terminal of the peptide; the opposite end is the C terminal



▲ **Figure 2.11** The formation and breakage of a peptide bond

Levels of organisation

▼ **Table 2.4** The levels of organisation of proteins

Level of organisation	Description	Comments	Structure
primary structure	• sequence of amino acids • position of disulfide bonds	determined by the gene that codes for the polypeptide position of cysteines in the sequence determines where these will form	
secondary structure	• α-helix • β-pleated sheet	polypeptide forms a right-handed helix polypeptide folds back and forth to form a flat sheet	
tertiary structure	further folding and coiling of polypeptide to give complex 3D shape	α-helices and β-pleated sheets as well as regions without any distinct secondary structure are present tertiary structure is stabilised by interactions between R-groups within the tertiary structure	
quaternary structure	two or more polypeptides associate together to form a protein	the polypeptides can be identical or different	

Bonds that stabilise proteins

There are four bonds that stabilise polypeptides:

- **hydrogen bonds** between polar groups, such as the dipolar –NH and –CO groups either side of the –C–N– bond of peptide bonds
- **ionic bonds** between ionised amine ($-NH^+$) and carboxyl ($-COO^-$) groups of R-groups

- **disulfide bonds** between the sulfur-containing R-groups of cysteine residues
- **hydrophobic interactions** between non-polar R-groups.

Globular and fibrous proteins

Globular proteins are folded into complex 3D shapes. Hydrophilic R-groups on their surface form hydrogen bonds with water so the molecules are soluble in water. Internally, amino acids with hydrophobic R-groups exclude water to form a hydrophobic interior. These are metabolic proteins because they carry out a range of functions that contribute to the metabolism of organisms. Fibrous proteins are insoluble in water and have simple shapes, such as a helix. They are structural proteins.

Haemoglobin is a globular protein found inside red blood cells; **collagen** is a fibrous protein that is a major component of the supporting material between cells known as the extracellular matrix. Both proteins are formed from more than one polypeptide.

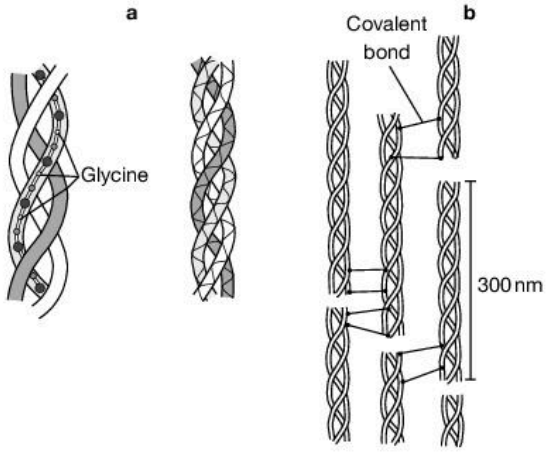
Structure and function of haemoglobin

Each molecule of haemoglobin is composed of four polypeptides: two **α-globins** and two **β-globins** (Figure 2.13). Each polypeptide has a tertiary structure consisting only of α-helices. In the centre of each polypeptide is a **haem group** with a central atom of ferrous iron (Fe^{2+}). Each haem group can form a temporary bond with an oxygen molecule. There are four haem groups, so each haemoglobin molecule can carry four molecules of oxygen. The addition of each molecule of oxygen changes the shape of haemoglobin making it easier to accept the next oxygen molecule. This is because the molecule changes shape opening out to expose the haem groups so they can accept oxygen.

Structure and function of collagen

Collagen is an extracellular protein made of three identical polypeptides that form left-handed helices. These are wound around each other to form a triple helix. The polypeptides are long (about 1000 amino acids) with glycine as every third amino acid. Glycine has the smallest R-group (–H) so it does not take up much space. This means the helices can be wound tightly together and form many hydrogen bonds between them (see Figure 2.14a).

Many triple helices are joined together by covalent bonds to form collagen fibrils which group together to form a collagen fibre (see Figure 2.14b). The ends of the triple helices do not coincide within each fibril so that there are no lines of weakness where the fibre may break. This structure makes collagen suitable for structures such as tendons, which connect muscles to bones, have a high tensile strength and resist pulling forces.



◀ **Figure 2.14 a** The triple helix of collagen; **b** triple helices are joined together to form collagen fibrils and then these form collagen fibres

Key terms

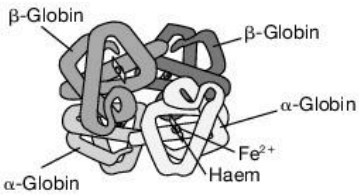
Globular protein: a metabolic protein that is soluble in water and has a spherical or near-spherical shape, e.g. haemoglobin, enzymes.

Fibrous protein: a structural protein that is insoluble in water and has a chain-like shape rather than a spherical shape.

Diagrams of haemoglobin sometimes make students think that each red blood cell contains one molecule of haemoglobin. It is estimated that there are about 280 million of them in each red blood cell.

★ Exam tip

Haem is an example of a **prosthetic group** – a part of a protein molecule that is not made of amino acids. Proteins with prosthetic groups are conjugated proteins.



▲ **Figure 2.13** Haemoglobin is a protein with all four levels of organisation. In this diagram you can see the quaternary structure composed of two α-globins and two β-globins

★ Exam tip

Cellulose is a polysaccharide and collagen is a protein, but both share similar structural features and functions. Try Question 6 (c)(i) in the Exam-style questions in this Unit.

Practical skills

Testing for biological molecules

You are likely to be examined on these tests for biological molecules in Paper 3. However, you can also expect questions on these tests in the other papers. You should learn the names of the reagents and the practical steps in each test.

Exam tip

The reagent used is iodine in potassium iodide solution (or shortened to iodine in KI solution). 'Iodine solution' may be used but *never* 'iodine'.

Test for starch The reagent used in the starch test is **iodine in potassium iodide (KI) solution** (known simply as iodine solution). The substance to be tested may be a solid or a liquid. Place solid samples on a white tile or in a Petri dish and liquid samples into a test-tube.

Use a dropping pipette to add iodine in potassium iodide solution.

Colour change in iodine in potassium iodide solution	Result	Explanation
yellow-orange to blue-black or blue	positive starch present	iodine binds to the centre of the helix of amylose to form a starch-iodine complex which has a blue-black colour
no change; iodine solution remains yellow-orange	negative no starch present	no starch for iodine to bind to

Test for reducing sugars For solid samples, make a solution in water (can be ground with a pestle and mortar and filtered). Put about 1 cm^3 of the test solution into a test-tube and add an equal volume of Benedict's solution. Heat to about 70–90°C in a water bath (do not heat directly with a Bunsen burner). Watch carefully for colour changes and the formation of a precipitate.

Colour change on heating with Benedict's solution	Result	Explanation
blue to green/yellow/orange/ (precipitate difficult to see unless allowed to settle) red with a precipitate	positive reducing sugars present (not necessarily glucose)	sugar reduces copper(II) ions (Cu^{2+}) in Benedict's solution to copper(I) ions (Cu^{+}) to form a precipitate of copper(I) oxide
no change; Benedict's solution remains blue	negative	no reducing sugars present to react with copper(II) ions

Test for non-reducing sugars The only common non-reducing sugar is sucrose (see page 14). Divide the test solution into two parts, **A** and **B**. Test **A** with Benedict's solution as above. Add a few drops of dilute hydrochloric acid to **B** and boil for at least three minutes. Cool the test-tube and add dilute sodium hydroxide solution or solid sodium hydrogen carbonate (beware, the latter will fizz). When neutralised, test with Benedict's solution as above.

Colour change on heating with Benedict's solution	Result	Explanation
A – no change **B** – blue to green/yellow/orange/ red precipitate	negative for reducing sugar, positive for non-reducing sugar	hydrochloric acid acts as a catalyst to hydrolyse sucrose to fructose and glucose that are both reducing sugars
A – no change **B** – no change	negative for both reducing and non-reducing sugars	no reducing sugars present even after using hydrochloric acid to hydrolyse any non-reducing sugars

Test for proteins If the substance to be tested is solid, make a solution in water. Place 1 cm^3 of the test solution in a test-tube. Add the same volume of **biuret reagent** and mix by shaking the tube gently from side to side.

Colour change with biuret solution	Result	Explanation
blue to violet/purple/lilac	positive	a coloured complex forms if there are peptide bonds in the test substance
no change to the blue colour	negative	no peptide bonds present

Test for lipids Lipids are insoluble in water, but they are soluble in organic solvents such as ethanol. Crush any solid material to be tested in a pestle and mortar and add some ethanol. If the test substance is a liquid, add some ethanol and shake to dissolve. Pour off the ethanol, which may have dissolved some lipids, into a test-tube of water (do not mix).

Change when adding ethanol to water	Result	Explanation
white cloudy (milky) – an emulsion	positive	the ethanol dissolves the lipid; on addition to water the lipid is dispersed throughout the water as tiny particles – an emulsion
no change	negative	no lipid present to be dispersed

Exam tip

You should carry out these tests for yourself in the lab. Learn all the details. Take photographs of the results to help you remember the practical procedures.

Semi-quantitative test for reducing sugars The final colour change with the Benedict's test indicates how much reducing sugar is present in a test sample. One way to improve this estimate is to make up a series of colour standards using a stock solution of glucose and Benedict's solution.

1. Take 20 cm^3 of the stock solution of known concentration, e.g. 100 $g\,dm^{-3}$.
2. Use the stock solution and distilled water to make the following dilutions:
   50.0, 20.0, 10.0, 5.0, 1.0, 0.5, 0.1 $g\,dm^{-3}$.
3. Place equal volumes of the dilutions into labelled test-tubes.
4. Carry out the Benedict's test as on page 20. Use the same volume of Benedict's solution in each test-tube and heat all the test-tubes for the same length of time.
5. Cool the test-tubes and keep them to use as colour standards.
6. Carry out the Benedict's test on a solution of the test substance using *exactly* the same procedure as when making the colour standards.
7. Cool the test-tube and place next to the colour standards to determine the concentration of reducing sugar; the answer may be a range, e.g. between 1.0 and 5.0 $g\,dm^{-3}$. Place a piece of white card behind the tubes to help match the colours.

Remember

Take photographs for a permanent record.

Raise your grade

1 (a) Triglycerides and phospholipids are similar in structure.

Describe how the structure of phospholipid molecules differs from triglyceride molecules. [3]

Phospholipids have only two fatty acid chains whereas triglycerides have three. ✔
Phospholipids have a hydrophilic phosphate group instead of one of the fatty acids. ✔
Phospholipids have hydrophilic heads and hydrophobic tails. ✔

This answer has three correct differences between these two groups of lipids.

(b) (i) Describe how to carry out a test to show the presence of triglycerides in samples of seeds of different species. [3]

The sample to be tested is put into a test-tube and dissolved in ethanol and shaken. ✔ The solution is then poured into another test-tube of water. ✔

Correct practical details, but the candidate has not stated what to look for if the test is positive. A complete answer would add that a white emulsion would form in the water. A good answer would also state to keep the samples from each species separate and test them separately.

(ii) Explain why the test you describe is a qualitative test. [1]

The result shows you about the quality of the substance tested. ✘

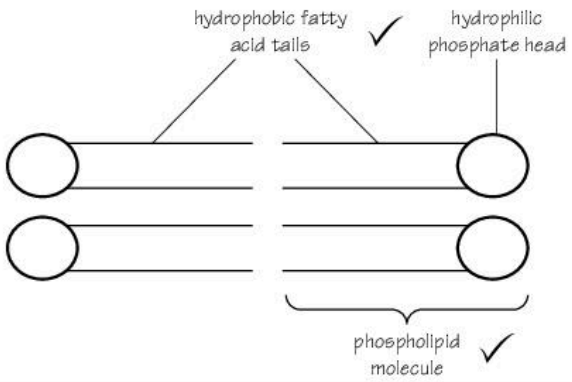
In this context, it is better to write that a qualitative test identifies the presence or absence of the substance; it is a description of what has been observed. It does not tell us how much of the substance is present. For that we need a quantitative test.

(c) Explain how the structure of triglycerides makes the molecules more suitable for long-term energy storage than glycogen. [3]

Triglycerides have three long fatty acid chains. These chains are composed of carbon and hydrogen. When they are respired they release far more energy than when the glucose from glycogen is respired. ✔

Not a complete answer. It is necessary to say that more energy for respiration is released from fat compared with the same *mass* of glycogen.

(d) Draw a diagram to show the arrangement of four phospholipid molecules in a cell membrane. [2]



The four phospholipid molecules are drawn correctly showing how they would appear in a phospholipid bilayer. To make the answer perfectly clear the candidate has labelled a phospholipid and shown the hydrophilic and hydrophobic regions.

2 (a) Plants produce monosaccharides such as glucose. Much of the glucose is converted into the storage compounds, amylose and amylopectin.

(i) State one place where amylose and amylopectin are found in plant cells. [1]

Starch grains in chloroplasts ✔

Correct answer.

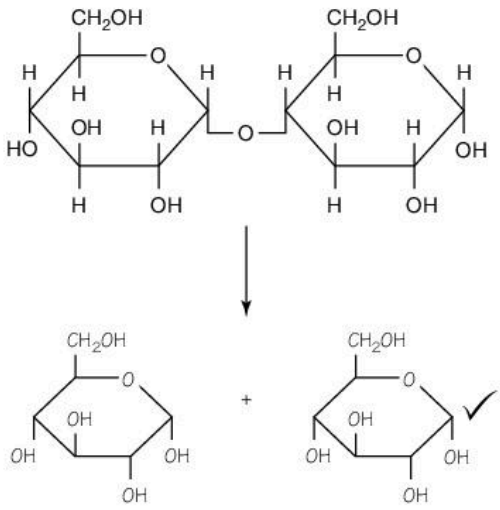
(ii) Describe how amylopectin differs from amylose. [2]

Amylopectin is branched but amylose is a straight chain. ✔

Incomplete answer. The candidate has not stated that amylopectin has α-1,6 glycosidic bonds as well as α-1,4 glycosidic bonds.

Maltose is the disaccharide produced during the breakdown of starch.

(b) (i) Complete the diagram below to show the reaction catalysed by maltase. [3]



The diagram shows that two α-glucose molecules are formed. The candidate should have included the water molecule that is the other reactant and also labelled one of the α-glucose molecules.

(ii) State the type of reaction that is catalysed by maltase. [1]

Hydrolysis. ✔

Correct answer.

(c) Maltose and glucose are both soluble in water. Explain how water acts as a solvent for molecules, such as maltose and glucose. [3]

Sugars are soluble in water because they are polar. ✔ Water is also polar so makes a good solvent for sugars.

Incomplete answer. Candidate should refer to the dipolar nature of each water molecule and the hydrogen bonding between the polar groups on the sugar molecules and water.

Exam-style questions

1 Haemoglobin is an example of a protein with

A primary structure only

B primary and secondary structure only

C primary, secondary and tertiary structure only

D primary, secondary, tertiary and quaternary structure [1]

2 Which row shows the correct bond associated with each of the biological molecules shown?

	Disaccharide	Polysaccharide	Protein	Triglyceride
A	hydrogen	glycosidic	ester	peptide
B	peptide	ester	glycosidic	hydrogen
C	glycosidic	glycosidic	peptide	ester
D	glycosidic	peptide	ester	glycosidic

[1]

3 Starch and glycogen are polysaccharides. Which is a feature of starch, but **not** of glycogen?

A Starch is made from α-glucose

B Starch has an unbranched component

C Starch contains 1,6 glycosidic bonds

D Starch contains 1,4 glycosidic bonds [1]

4 Some students made three statements about the primary structure of a polypeptide.

1 The primary structure is the sequence of amino acids of the polypeptide.

2 The primary structure is stabilised by hydrogen bonds.

3 The primary structure determines the tertiary structure of the polypeptide.

Which of these statements is/are true?

A 1, 2 and 3; B 1 and 2 only;
C 1 and 3 only; D 1 only [1]

5 Which features is/are found in a phospholipid molecule?

1 ester bonds

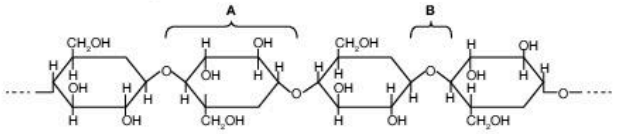
2 three fatty acid chains

3 peptide bond

4 hydrophilic group

A 1, 2, 3 and 4; B 1 and 4 only;
C 2 and 3 only; D 4 only [1]

6 (a) The figure shows part of a molecule of cellulose.



(i) Identify the monomer indicated by **A**. [1]

(ii) State the name of the bond indicated by **B**. [2]

(iii) State the name of the type of reaction which forms the bond labelled **B**. [1]

(b) Describe the properties of cellulose that make it suitable as the main component of the cell walls of plant cells. [5]

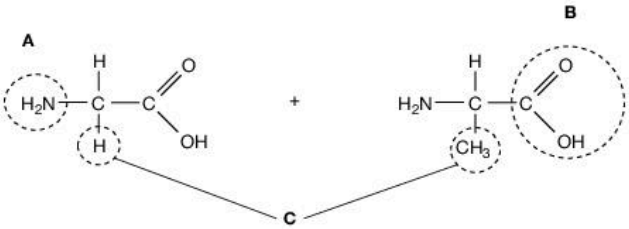
(c) Collagen is a fibrous protein.

(i) State **three** ways in which the structure of a collagen molecule differs from the structure of a cellulose molecule. [3]

(ii) Explain how a globular protein differs from a fibrous protein. [2]

[Total 14]

7 (a) The figure shows the two amino acids glycine and alanine.



(i) Name the parts of the molecules labelled **A**, **B** and **C**. [3]

(ii) Draw a diagram to show how the two amino acids are joined together by a condensation reaction. [3]

(iii) Name the bond that forms between the two amino acids. [1]

(iv) State the location in the cell where amino acids are joined together in reactions like the one you have drawn. [1]

(b) A protein composed of a single polypeptide has no disulfide bonds. Explain how the tertiary structure of this protein is stabilised. [4]

(c) Explain why haemoglobin is said to have quaternary structure. [1]

3 Enzymes

Key points

- ☐ Explain that enzymes are globular proteins that catalyse metabolic reactions.
- ☐ State that enzymes function inside cells (intracellular enzymes) and outside cells (extracellular enzymes).
- ☐ Explain the mode of action of enzymes with respect to their structure, specificity and role in lowering activation energy.
- ☐ Carry out a practical investigation into the progress of an enzyme-catalysed reaction.
- ☐ Carry out practical investigations to determine the effects of temperature, pH, substrate concentration, enzyme concentration and inhibitor concentration on enzyme-catalysed reactions.
- ☐ Explain the influence of temperature, pH, substrate concentration, enzyme concentration and inhibitor concentration on enzyme-catalysed reactions.
- ☐ Explain that the maximum rate of reaction (V_{max}) is used to derive the Michaelis–Menten constant (K_m) which is used to compare the affinity of different enzymes for their substrates.
- ☐ Explain the effects of competitive and non-competitive inhibitors on the rate of enzyme activity.
- ☐ Carry out practical investigations to compare the activity of an immobilised enzyme with its activity when free in solution.
- ☐ Explain the effect of immobilising enzymes on their activity.

The mode of action of enzymes

Enzymes are catalysts

In a reaction, **substrate** molecules are converted into molecules of **product**. Most reactions are reversible and **catalysts**, which are effective in very small quantities, speed up both the forward reaction and the reverse reaction:

$$A + B \rightleftharpoons^{\text{catalyst}} C + D$$

Catalysts cannot change the equilibrium that exists between substrate concentration and product concentration, but they can increase the rate at which the equilibrium is achieved.

Enzymes are biological catalysts. Almost all enzymes are proteins that have a globular structure which provides an active site where the substrates of the reaction fit together closely, making the reaction more likely to occur.

Enzymes are either:

- **extracellular** – act *outside* cells; for example, in the lumen of the mammalian gut, or
- **intracellular** – act *inside* cells; for example, inside mitochondria.

Enzymes are specific

Two ways in which enzymes catalyse reactions by providing a place for substrates to fit:

- **lock and key model**: the shape of the **active site** is always **complementary** to the substrate and does not change shape when the substrate enters.

Key terms

Substrate: a substance that is changed by an enzyme during an enzyme-catalysed reaction.

Product: a substance that is the result of an enzyme-catalysed reaction.

Catalyst: a substance that increases the rate of a reaction without being used up itself.

Link

See Unit 6 for the production of proteins, such as enzymes, and Unit 1 for the role of different cell structures in synthesising and exporting proteins from cells.

AS Level

- induced fit model: the shape of the active site is not complementary to the substrate until it binds to the enzyme. It then changes shape to mould itself around the substrate so there is a better fit between the two molecules.

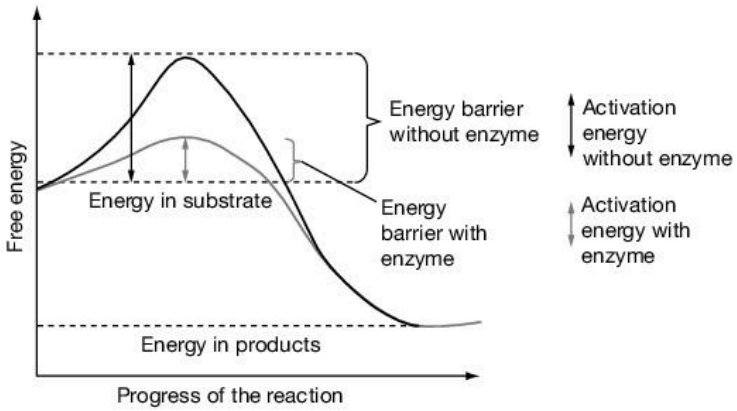
The **specificity** of an enzyme is determined by the shape of its active site, which must be complementary to the shape of the substrate.

Active sites

An active site has a 'pocket', 'depression' or 'groove' shape that is determined by the tertiary structure of the enzyme molecule. The folding of the polypeptide brings specific R-groups of amino acids together to form the appropriate shape for the substrate to fit tightly. The R-groups of some amino acids making the active site are adjacent to each other in the primary sequence, some are not. Substrate molecules are held in place within the active site by hydrogen bonds, ionic bonds or hydrophobic interactions, depending on the type of substrate. The combination of enzyme and substrate is an **enzyme–substrate complex**. When the substrate molecule is bound to the active site, the molecule is put under strain so that bonds are more likely to break or to form.

Activation energy

The reactions catalysed by enzymes often involve the formation or breaking of stable covalent bonds, which requires much energy. Enzymes provide a pathway in active sites where substrate molecules are positioned so that the activation energy is decreased (Figure 3.1).



▲ **Figure 3.1** Activation energy for an exothermic reaction

In Figure 3.1, the energy of the substrate is higher than the energy of the products, so the reaction is exothermic as it releases energy. Many of the reactions of respiration are like this. If the energy of the substrate(s) is lower than the energy of the product(s), the reaction is endothermic as energy is required from the surroundings. In many biochemical reactions the energy for these reactions is provided by ATP. Examples are starch and protein synthesis.

In some specialised human cells, for example liver cells, there are thought to be over a thousand different enzymes that carry out many metabolic reactions.

Remember

The tertiary structure of a polypeptide may consist of α-helices, β-pleated sheets and regions with no distinct secondary structure folded into a specific 3D shape stabilised by hydrogen bonds and other interactions between R-groups. See Table 2.4 on page 18 to remind yourself about the levels of organisation of proteins.

Exam tip

A substrate molecule fits into an active site because it has a complementary shape. Do not write that is has the *same shape* as the active site.

Key terms

Lock and key model: the active site of an enzyme and the substrate fit together like a 'lock' (enzyme) and 'key' (substrate) during an enzyme-catalysed reaction.

Active site: part of an enzyme molecule where the reaction takes place.

Induced fit model: the active site of an enzyme moulds itself so there is a complementary fit to the shape of the substrate during an enzyme-catalysed reaction.

Activation energy: the energy that must be overcome before a reaction can proceed.

Exam tip

Make a diagram similar to Figure 3.1 to show the role of an enzyme in an endothermic reaction.

Practical skills

Following the course of enzyme-catalysed reactions

Different enzymes change substrate to product at different rates. The **activity** of an enzyme is determined by finding the rate at which a reaction proceeds. This can be done by following the decrease in concentration of the substrate or the increase in the concentration of the product. During a reaction with fixed quantities of enzyme and substrate, the number of substrate molecules decreases, so the chance of a substrate molecule entering an active site decreases. This means that the number of product molecules formed decreases.

The breakdown of protein

You can follow the course of an enzyme-catalysed reaction by seeing how long it takes to reach an **end point**, which is something that can be observed or detected. For example, a solution of milk powder is cloudy. Add a protease (e.g., trypsin or pepsin) and the cloudiness gradually disappears because the protease catalyses the hydrolysis of protein in the milk. This reaction can be observed as follows:

1. Add 10 cm^3 of a solution of milk powder to test-tube 1.
2. Add 1 cm^3 of a protease solution to test-tube 2.
3. Place both test-tubes in a thermostatically controlled water bath at 25 °C for five minutes.
4. After five minutes, pour the contents of test-tube 2 into test-tube 1, return test-tube 1 to the water bath and start a timer.
5. Observe carefully and time how long it takes for the cloudiness to disappear.

Exam tip

Keeping the enzyme solution and substrate solution separate until they are both at the desired temperature for the reaction is called **equilibration**.

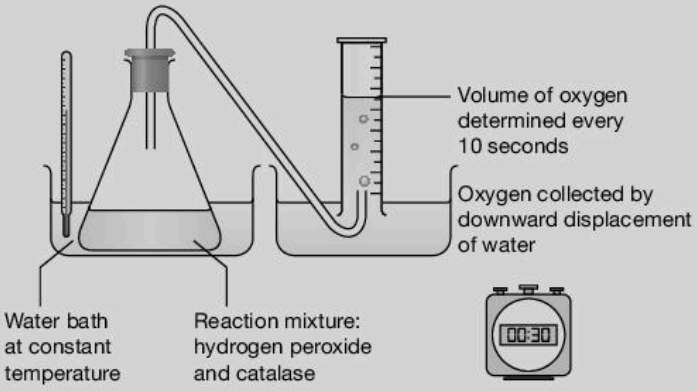
The time taken for the reaction to occur is not the rate of reaction. When there is a fast rate of reaction then the reaction will be completed in a short time. If the rate of reaction is slow, then the reaction will be completed in a long time. The rate of reaction is calculated as the reciprocal of the time taken: $\frac{1}{t}$ in which t = the time taken to reach the end point in seconds. The unit is seconds^{-1} (s^{-1}).

The breakdown of hydrogen peroxide

To follow the appearance of a product you can use the enzyme catalase, which catalyses the decomposition of hydrogen peroxide:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

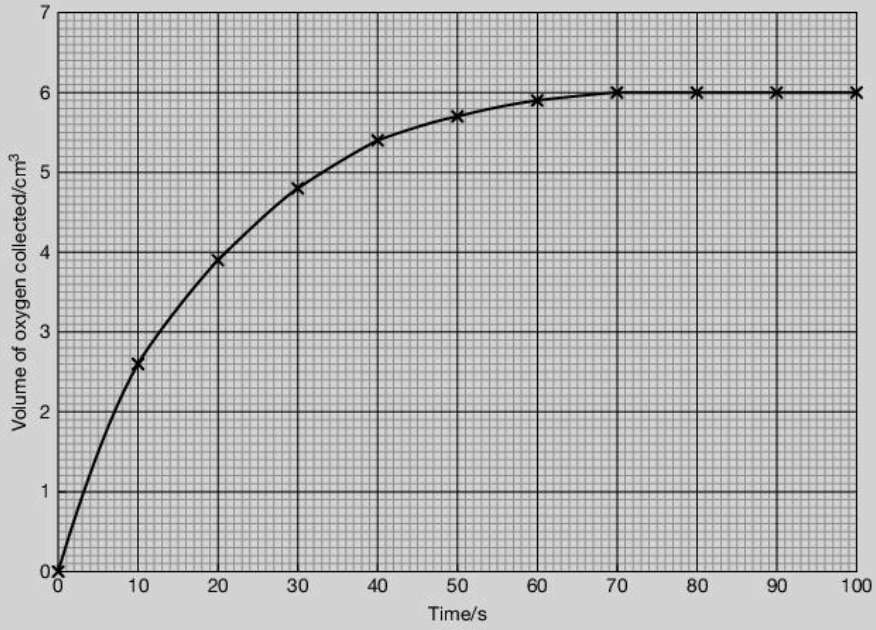
A solution of catalase could be used, but usually the enzyme is extracted from plant material, such as potato, celery or lettuce, that contains it. The apparatus can be set up as shown in Figure 3.2. A gas syringe could also be used to collect the oxygen produced.



▲ **Figure 3.2** Apparatus for following the decomposition of hydrogen peroxide to oxygen and water

Hydrogen peroxide is usually provided as a 20 volume solution; '20 volume' means that when 1 cm^3 of hydrogen peroxide is decomposed, 20 cm^3 of oxygen are produced. A 20 volume solution of hydrogen peroxide has a molarity of 1.67 $mol\,dm^{-3}$.

A set of results obtained for the breakdown of hydrogen peroxide are shown in the table. These have been plotted on a graph (Figure 3.3).



Time/s	Volume of oxygen collected/cm³
0	0.0
10	2.6
20	3.9
30	4.8
40	5.4
50	5.7
60	5.9
70	6.0
80	6.0
90	6.0
100	6.0

▲ **Figure 3.3** The volumes of oxygen produced after a solution of catalase is added to hydrogen peroxide

The rate is fastest at the beginning and decreases with time (Figure 3.3) because over time hydrogen peroxide is converted to the products of the reaction and so the concentration of the substrate decreases. When no substrate is left, the reaction stops. The collisions between enzyme molecules and substrate molecules are most frequent at the beginning of the reaction when the enzyme is added to the substrate.

Various factors affect the rate of enzyme activity

There are five factors that influence the activity of enzymes:

- substrate concentration
- temperature
- pH
- enzyme concentration
- inhibitor concentration.

★ Exam tip

Some of these factors influence the frequency of collisions between enzyme molecules and substrate molecules and some influence the stability of the tertiary structure of the enzyme molecule. See page 18 for more about this.

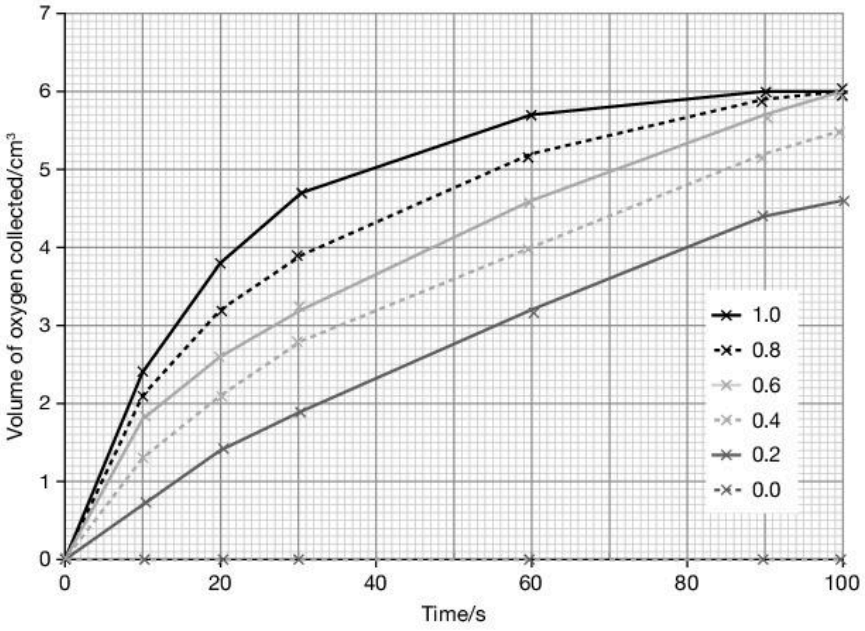
Substrate concentration

The procedure in Figure 3.2 is used to investigate the effect of substrate concentration. The procedure is repeated for each substrate concentration.

The results for a range of hydrogen peroxide concentrations are shown in Figure 3.4.

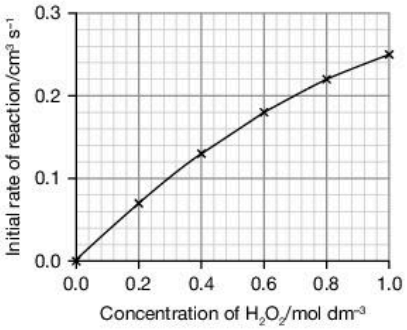
★ Exam tip

Note that it is the *concentration* of hydrogen peroxide that is increased, not the *volume* that is added to each reaction mixture. Avoid using 'amount', which often means 'volume' or quantity. Unit 20 Practical assessment page 233 has more about concentration.



▲ **Figure 3.4** The progress of the reaction with different concentrations of hydrogen peroxide. The key shows the concentrations of hydrogen peroxide in mol dm^{-3}

To determine the initial rate of reaction when the substrate concentration is at its highest, tangents are drawn to the curves. The rates are then plotted on another graph – see Figure 3.5.



▲ **Figure 3.5** How increasing the concentration of substrate influences the initial rate of the decomposition of hydrogen peroxide by catalase

As the substrate concentration increases to 1.0 mol dm^{-3}, the rate of reaction increases (Figure 3.5). After 0.8 mol dm^{-3} the rate remains constant at 0.2 $cm^3 s^{-1}$.

At a concentration of zero there was no reaction, so the line must start at the origin. The rate increases as more substrate molecules are available and there are more successful collisions with the active sites of the catalase molecules. At the concentrations shown in Figure 3.5, enzyme activity is limited by the concentration of substrate, because if the concentration is increased the rate increases. At concentrations greater than 1.0 mol dm^{-3} the rate remains constant. It is not limited by the substrate concentration because increasing the concentration has no effect. The rate must be limited by something else, such as enzyme concentration.

Exam tip

Always look carefully at the labels on the axes of graphs. The *x*-axis of Figure 3.5 is 'concentration of substrate'. It is *not* 'time'. The graph has the same shape as the time-course graph in Figure 3.3. A time-course graph shows what happens in one reaction mixture.

Link

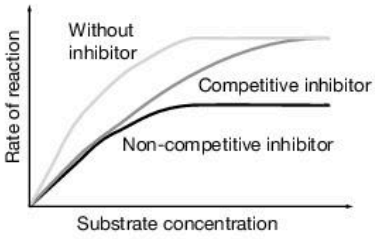
There is more about determining initial rates of reaction in Unit 20 Practical assessment page 237.

Here *limit* and *limiting* mean that some factor is preventing the rate of reaction from going any faster. It is as if the enzyme could work faster if it were provided with more substrate, but no more is available.

Inhibitors

Some substances interact with enzymes and reduce their activity. These are known as inhibitors.

- **Competitive inhibitors** fit temporarily inside the active site without being changed in a reaction. They compete with the substrate for entry to the active site. If the concentration of the substrate is increased then the effect of the inhibitor can be reduced (see Figure 3.6).
- **Non-competitive inhibitors** combine temporarily with parts of the enzyme molecule *other than* the active site. They do not occupy the active site, but bind to another site on the enzyme, known as an allosteric site. In response, the enzyme molecule changes its overall shape and the active site is no longer complementary to the substrate. Enzyme–substrate complexes cannot form, so the enzyme is inhibited. The effect of non-competitive inhibitors cannot be reduced by increasing the substrate concentration because substrate molecules cannot fit into the active site.



▲ **Figure 3.6** The effects of a competitive inhibitor and a non-competitive inhibitor on the rate of reaction of an enzyme for increasing substrate concentrations

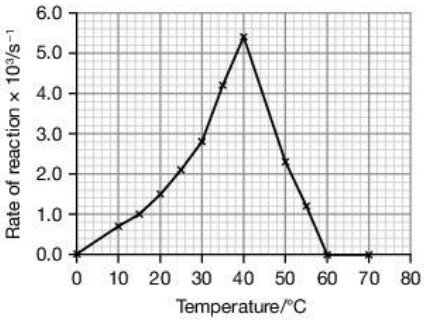
Figure 3.6 shows the effect of adding a fixed concentration of the two types of inhibitor.

The inhibitors described here are reversible inhibitors. The enzyme is not permanently disabled by the inhibitor. If the inhibitor is removed, then the enzyme becomes active again. You do not need to know about non-reversible inhibitors, but they are sometimes used in practical work.

Temperature

The activity of enzymes at different temperatures can be investigated by placing the reaction mixtures in water baths at a range of temperatures. The substrate and enzyme solutions are equilibrated at the temperatures used, then mixed together and kept at each of those temperatures over the range chosen. Figure 3.7 shows the effect of temperature on the hydrolysis of milk protein by a protease.

The data from this investigation are processed by calculating $\frac{1}{t}$ and multiplying the answers by 1000 (10^3). This is to give whole numbers which are much easier to plot on the graph in Figure 3.7.



▲ **Figure 3.7** The effect of increasing temperature on the rate of an enzyme-catalysed reaction. More results between 35 and 55 °C are needed to find the optimum temperature

The rate of reaction increases up to a maximum rate of 5.4×10^3 s^{-1} at a temperature of 40 °C. At temperatures above 40 °C the rate decreases steeply and there is no activity at 60 °C or above.

The rate increases because there is an increase in kinetic energy and the substrate molecules collide more frequently with the enzyme molecules. However, as the temperature increases, the enzyme molecules vibrate and the bonds stabilising the tertiary structure begin to break. At the optimum temperature, the number of successful collisions is at its maximum, but above that temperature more and more enzyme molecules become non-functional as they denature. At 60 °C all the enzymes are denatured.

Exam tip

A thermostatically controlled water bath is the best way to maintain a constant temperature in investigations with enzymes. This avoids having to use a thermometer to check the temperature of a beaker of water and continually adding hot or cold water.

Link

Look at page 18 to remind yourself about the bonds that stabilise the tertiary structure of proteins.

Enzyme concentration

The effect of increasing enzyme concentration can be investigated by making a simple dilution of a catalase solution (see page 234). The initial rates of reaction can be determined as described on page 237.

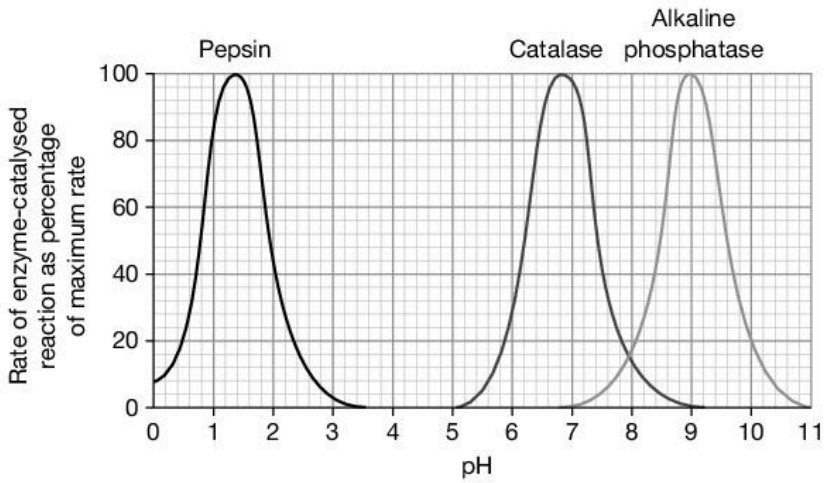
As the concentration of enzyme increases, the rate of reaction increases. The rate is directly proportional to the enzyme concentration. If there are more enzyme molecules, there will be more successful collisions per unit time. This assumes that at each concentration of enzyme, the substrate concentration is in excess so that substrate concentration does not limit the rate of reaction.

pH

Reaction mixtures can be prepared using special solutions – buffer solutions – to maintain a constant pH. It is possible to make buffer solutions for various ranges of pH. Different ranges of pH were used to investigate three enzymes:

- pepsin
- catalase
- alkaline phosphorylase.

The rates of reaction for each enzyme were determined and then expressed as a percentage of the maximum rate as shown in Figure 3.8.



▲ **Figure 3.8** The activity of three enzymes at different values of pH. The reaction mixtures were kept at 20 °C

pH is the measurement of the hydrogen ion concentration in a solution. At the optimum pH, the enzyme is at its most active as the active site has the correct shape, which is determined by interactions between the R-groups of amino acids in the active site. As the hydrogen ion concentration changes, some of these interactions break and the active sites become less effective. At certain values of pH, enzymes change their shape so much that they are **denatured**. At values of pH just above and below the optimum, enzymes change shape slightly but are still active. This is partial denaturation and is reversible if the pH returns to the optimum.

As pH is a factor that affects the activity of enzymes, it is important to keep the pH constant when investigating *other* factors, such as temperature, substrate concentration and enzyme concentration. You can do this by finding a suitable pH for the enzyme that you are investigating and using a buffer solution that will keep the pH constant in each

★ Exam tip

Converting the actual rates of reaction into 'percentage of maximum rate' makes it easier to compare the effect of pH on these three enzymes.

Look at the graph and follow these descriptions:

- each enzyme is active over a range of pH
- as pH increases, the activity of each enzyme increases to a maximum
- maximum activity occurs at the optimum pH for each enzyme
- above the optimum pH, the activity decreases
- there is no activity over certain ranges of pH, e.g. < pH 5 and > pH 9 for catalase.

★ Exam tip

If you are investigating one of the factors that affect enzyme activity, then the other three must be kept constant, otherwise you will not be able to make valid conclusions about the factor you are investigating.

reaction mixture for as long as the reaction lasts. Variables that are not changed during an investigation but which are kept constant are called **controlled variables**.

The five factors you have considered can influence the following aspects of these models of enzyme action:

- temperature, substrate concentration and enzyme concentration influence the **frequency of collisions** between enzyme molecules and substrate molecules
- temperature, pH and inhibitors affect the **stability of enzyme molecules**; substrate molecules can only enter active sites if the tertiary structure is not disrupted.

Worked example

a) Use the information in Figure 3.8 to complete the table.

Enzyme	Range of pH over which enzyme is active	Optimum pH
pepsin		
catalase		
alkaline phosphorylase		

b) **Explain** why buffer solutions are used when investigating enzyme activity.

c) Suggest how you could check that the pH does not change in a reaction mixture even though a buffer solution has been added.

d) Explain fully why the temperature of the enzyme solutions and protein solution were kept at 20 °C for ten minutes before they were mixed together.

Answers

a) Pepsin: pH range 0–3.5; optimum pH 1.5.
Catalase: pH range 5–9; optimum pH 6.8.
Alkaline phosphorylase: pH range 7–11; optimum pH 9.0.

b) A buffer solution maintains a constant pH. pH is a factor that affects the activity of enzymes. Ionic bonds and hydrogen bonds within protein molecules are broken when the pH changes. These bonds stabilise the tertiary structure of the enzymes. The breakage of these bonds causes the active site to lose its specific shape. Substrate molecules cannot fit into the active site so the reaction does not occur.

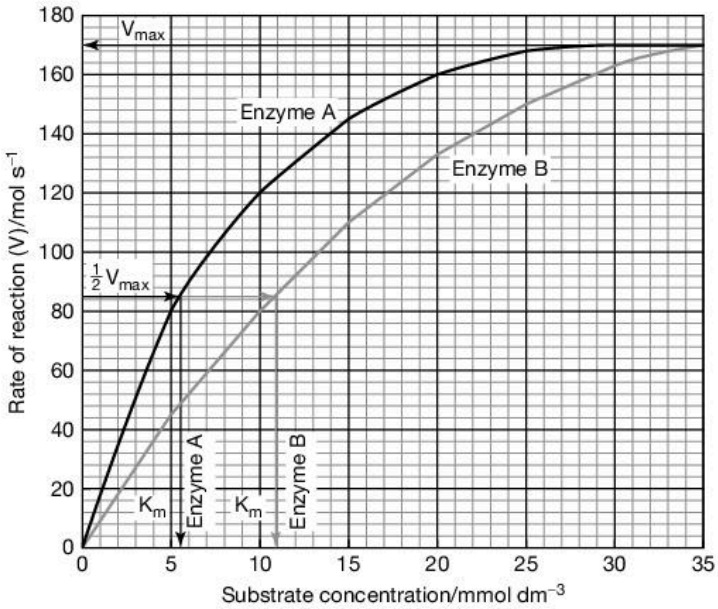
c) *Either* take samples and test with a pH indicator solution or pH papers (e.g. universal indicator solution or universal indicator papers) *or* use a pH meter and pH probe to monitor any changes in the reaction mixtures. Test the pH throughout from time 0 until the time when the reaction is complete.

d) *The solutions of the enzymes and the protein solution were equilibrated*

Temperature is a factor that influences the rate of enzyme-catalysed reactions. If the temperature of the solutions was not at 20 °C when they were mixed together, the rate would change over time. The temperature would change over the time period when results would be taken to derive the initial rate of reaction. The results would not be valid as the temperature has not been kept constant.

Rates of reaction and the Michaelis–Menten constant

Different enzymes have different affinities for their substrates. The term **affinity** in this context means the ease with which the substrate fits into the active site. The **Michaelis–Menten constant (K_m)** is a measure of this affinity. The K_m for any enzyme is determined by measuring the rate of reaction at different concentrations of substrate. As we have seen, at a certain concentration the rate remains constant because enzyme concentration becomes the limiting factor. This maximum rate is known as the V_{max} in which V represents velocity (speed) of the rate of reaction. The Michaelis–Menten constant (K_m) is determined by calculating the substrate concentration at half the value of V_{max}. You can see this for two enzymes in Figure 3.9.



▲ **Figure 3.9** Determining the Michaelis–Menten constant (K_m) for two enzymes, A and B. V is the reaction velocity (rate of reaction per unit time) and V_{max} is the maximum velocity of the reaction

Remember that the graph in Figure 3.9 is a plot of the *initial rates of reaction* for different concentrations of substrate. In the example, the range of concentrations is 0–35 mmol dm^{-3}. At the maximum rate, every active site is constantly being filled as the reaction proceeds. This is the *maximum number of enzyme–substrate complexes* (ESCs) that can be formed by the enzyme under the conditions of the reaction.

Enzyme A reaches V_{max} at a lower concentration than enzyme B. Enzyme A forms the maximum number of ESCs at a *lower substrate concentration* than enzyme B. This means that A has a *higher affinity* for the substrate than enzyme B. Enzymes with high affinity for their substrate have a low value for K_m while enzymes with a low affinity have a high value for K_m.

A low value for K_m indicates that the enzyme requires only a small amount of substrate for their active sites to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations. A high value for K_m indicates the need for high substrate concentrations to achieve maximum reaction velocity.

Key terms

Michaelis–Menten constant (K_m): the substrate concentration at which an enzyme functions at half its maximum rate ($½V_{max}$). This is a measure of the affinity of an enzyme for its substrate. K_m is inversely related to the affinity.

V_{max}: the maximum velocity (V) or rate of a reaction which occurs at a certain concentration of substrate for any enzyme under fixed conditions of pH and temperature.

Exam tip

Some enzymes catalyse several different reactions and act on different substrates. K_m is a way to compare the affinity of an enzyme for its different substrates. K_m is also used to determine the effect of inhibitors on an enzyme.

Exam tip

You can describe the importance of K_m by stating that it is inversely related to the affinity of the enzyme for its substrate.

Remember

The conditions of the investigation displayed in Figure 3.9 must be the same for each enzyme to make a valid comparison between their values of K_m. Temperature and pH must be the same, for example.

Worked example

Competitive inhibitors and non-competitive inhibitors have different effects on enzyme activity. They differ in their effects on the maximum velocity of an enzyme-catalysed reaction (V_{max}) and on the Michaelis–Menten constant (K_m).

a) Sketch a graph to show the effect of a competitive inhibitor on the activity of an enzyme.

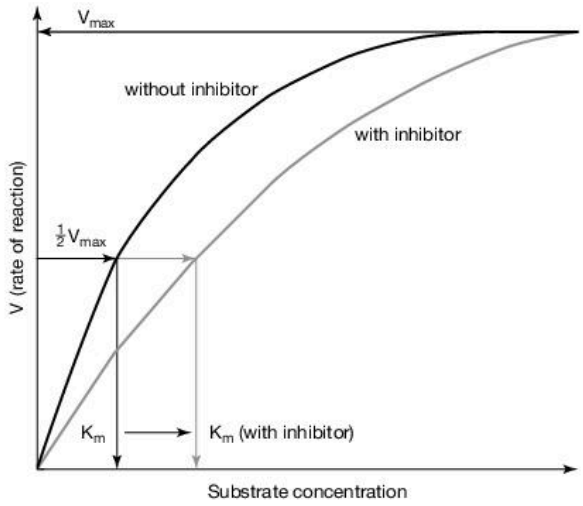
b) Use your sketch graph to show the effect of the competitive inhibitor on V_{max} and K_m.

c) Sketch a second graph to show the effect of a non-competitive inhibitor on the activity of an enzyme.

d) Use your sketch graph to show the effect of the non-competitive inhibitor on V_{max} and K_m.

Answers

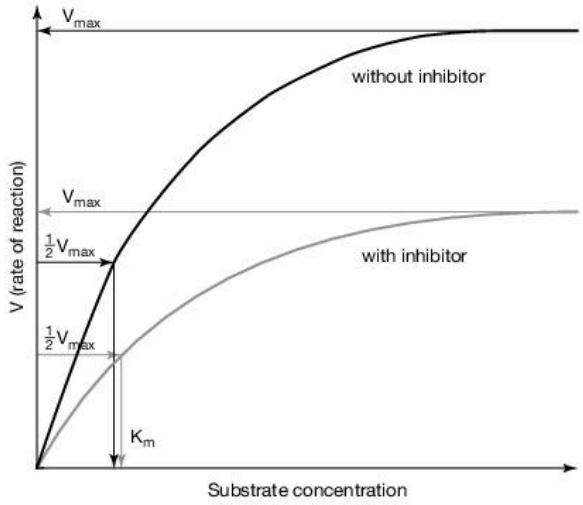
a)



Marks are awarded for putting the substrate concentration on the *x*-axis and the rate of reaction or velocity (V) on the *y*-axis; for both lines (with and without inhibitor) reaching the same maximum rate; and for the line showing the effect of the inhibitor being to the right of the line for 'without inhibitor'.

b) Marks are given for showing that the competitive inhibitor has no effect on V_{max} but it increases the K_m so that the enzyme has a lower affinity for its substrate when the inhibitor is present. (This is because the inhibitor molecules occupy the active site so that substrate molecules cannot enter. The effect of this competition is overcome as the substrate concentration is increased.)

c)



Marks are awarded for putting the substrate concentration on the *x*-axis and the rate of reaction or velocity (V) on the *y*-axis; for the line for 'without inhibitor' reaching a higher maximum rate than the line for 'with inhibitor'; and for the lines having the same shape.

d) Marks are awarded for showing that the non-competitive inhibitor reduces V_{max} but the K_m remains the same.

Immobilised enzymes

In many industrial processes when enzymes are used in solution with their substrates, it is difficult to recover them after the reaction they catalyse is finished. If the enzymes are **immobilised** inside gelatinous (jelly-like) beads, it is easy to recover them from reaction mixtures to reduce wastage. Also, the beads can be packed into columns so that substrate solutions are added at the top and product is available at the bottom. One way to immobilise enzymes is within beads of calcium alginate. The substances used to immobilise enzymes and cells are insoluble and inert.

The properties of immobilised enzymes are often different to their properties when they are free in solution. For example, the frequency of collisions between substrate and enzyme decreases as substrate molecules have to diffuse through the beads to the active sites of the enzymes. However, the beads provide protection to the enzymes so they are more stable at higher temperatures and are active over wider ranges of pH. The tertiary structures of enzymes are more stable inside beads than outside if the pH and/or temperature changes.

Key term

Immobilised enzyme: enzymes that are not free in solution as they are trapped inside a bead or attached to a surface of a bead or other sort of surface.

Cells can also be immobilised in calcium alginate beads. Immobilised yeast can be used as a source of catalase and sucrase in practical investigations.

Practical skills

Making immobilised catalase

A solution of an enzyme is mixed with an equal volume of sodium alginate solution. The mixture is added to a calcium chloride solution. When the sodium alginate comes into contact with calcium chloride it forms a gel enclosing the molecules of enzyme in small beads.

Any enzyme can be immobilised using this technique. Catalase, usually from plant (e.g. lettuce or celery) or animal tissue, is often used in practical work. Plant tissue can be put into a blender with water for several minutes, the tissue is filtered and the filtrate contains catalase molecules released from the cells.

Enzymes can be immobilised in calcium alginate beads by following this procedure.

1 Pour about 100 cm^3 of calcium chloride solution into a measuring cylinder.

2 Using a fine pipette drip approximately 10 cm^3 of the catalase and sodium alginate mixture from a height of about 20 mm above the top of the cylinder. Maintain a gentle pressure on the pipette to avoid introducing air bubbles into the mixture.

3 As soon as the drops of mixture enter the calcium chloride solution they form into beads. When you have made enough beads, pour off the calcium chloride solution and transfer the beads to a small beaker. Place the beads into a beaker of water until required.

4 The beads can be placed into test-tubes of hydrogen peroxide solution. They usually sink into the test-tube and then rise again to the surface when bubbles collect around the beads.

The beads can be used in investigations to find out the effect of temperature, pH, substrate concentration and, by making several batches of beads with different volumes of catalase added to sodium alginate, enzyme concentration. The effect of immobilising catalase can be investigated by making a solution of the enzyme *at the same concentration* and seeing how the rates of reaction compare.

Link

Raise your grade question 2 is about immobilised enzymes.

Raise your grade

1 Intracellular enzymes can be found in a number of locations within plant cells.

(a) State three places in a plant cell where intracellular enzymes can be found. [3]

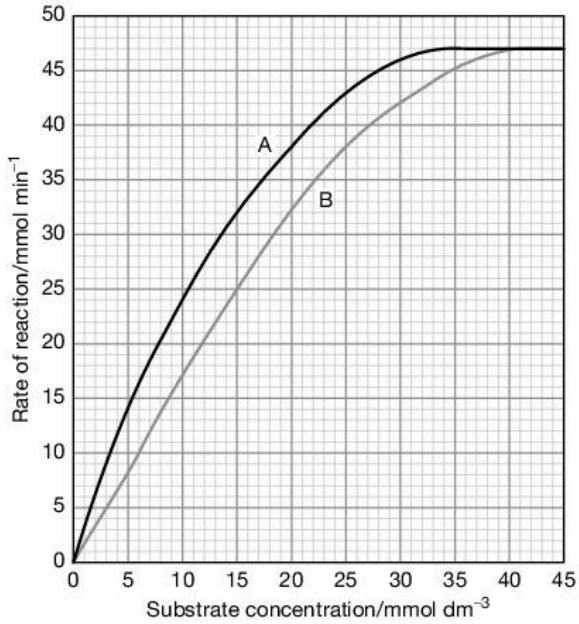
The three places are: mitochondria, ✔ nucleus ✔ and chloroplasts. ✔

Correct answers.

The intracellular enzyme dopa oxidase is found in the cells of some plants.

Dopa oxidase catalyses a reaction that gives a colour change. The effect of an inhibitor on the rate of reaction at different substrate concentrations was investigated. The graph shows the results.

Line A shows the results *without* the inhibitor. Line B shows the results *with* the inhibitor.



(b) Use the graph to determine:

(i) the maximum rate of reaction (V_{max}) for line A. [1]

47 mmol min^{-1}. ✔

(ii) the Michaelis–Menten constant (K_m) for line A. [1]

10 mmol dm^{-3} ✔

(iii) the effect of the inhibitor on K_m of this enzyme. [1]

The K_m is increased to 14.0 mmol dm^{-3} ✔

(iv) the effect of the inhibitor on V_{max}. [1]

V_{max} is unchanged ✔

Correct answers to all parts of (b).

(c) A different enzyme has a much higher K_m than dopa oxidase. What does this tell you about the two enzymes? [1]

The enzyme with the higher K_m has a lower affinity for its substrate than dopa oxidase. ✔

Correct answer.

(d) Identify the type of inhibitor and give a reason for your answer. [2]

The inhibitor is competitive. ✔ This is because it competes with the substrate for the active site. ✘

No reason given based on the evidence in the graph. Answer should state that at high concentrations the substrate competes effectively with the inhibitor so V_{max} is the same as in the reactions without the inhibitor.

2 This question is about immobilised catalase (see the Practical skills feature on page 35).

A student is set the task of planning an investigation into the effect of substrate concentration on the activity of immobilised catalase.

(a) State the independent variable and the dependent variable for this investigation [2]

independent variable concentration of hydrogen peroxide, ✔ *dependent variable* volume of oxygen collected ✔

Correct answers.

(b) Explain how you will change the independent variable. [3]

Make up at least five solutions ✔ of hydrogen peroxide by diluting a 20 vol (6 %) solution with water. The concentrations will be between 0 and 20 vol. ✔

A partial answer. The actual concentrations are not included and details of the dilution process are also not given. The concentrations should be equally spaced between 0 and 20 vol, so 0.0, 5.0, 10.0, 15.0 and 20.0 would be the minimum acceptable.

(c) Explain how you will measure the dependent variable. [2]

Collect the gas (oxygen) produced ✔ in a flask that is marked with volumes in ml.

Correct idea, but more detail is expected, e.g. candidate should indicate that the volumes should be read and recorded at set intervals of time. Most apparatus is marked with the volume in ml (millilitres), but exam papers use the unit cm^3 and this is the unit you should use in your answers.

(d) Explain how you will process and present the results. [3]

The time taken to collect a known volume (e.g. 20 cm^3) could be recorded. The time taken could be used to calculate a rate as $1/t$. ✔ The rates for each concentration are plotted on a graph and a line of best fit drawn between the points. ✔

This is a correct way to process the results. Another is to use the same technique as in Figures 3.5 and 3.6 by recording the volumes at intervals and plotting graphs to show the progress of the reaction at *each concentration*. The initial rate for each concentration is calculated by taking a tangent (see Unit 20 Practical assessment page 237) instead of calculating $\frac{1}{t}$. The results should be presented as a line graph with a line of best fit.

Exam-style questions

1 An intracellular protease acts on cell proteins that are no longer required by a cell.

Which bonds are broken by this protease?

A disulfide

B ester

C glycosidic

D peptide [1]

2 A student investigated the effect of amylase on the breakdown of starch. Three steps were included in the procedure.

1 The amylase solution and the starch solution were kept at 25 °C for five minutes before mixing.

2 Samples were removed from the reaction mixture and tested with iodine in KI solution at 30 s intervals.

3 The reaction mixture was kept stirred throughout.

Which step or steps ensured that variables were controlled?

A 1, 2 and 3 B 1 and 2 only

C 2 and 3 only D 1 only [1]

3 A student prepared some test-tubes with solutions as follows:

Test-tube	Contents
1	starch and α-amylase
2	glucose and sucrase
3	glycogen and α-amylase
4	starch and sucrase
5	sucrose and amylase

All the test-tubes were kept at 40 °C for 15 minutes. In which test-tubes would reducing sugar be detected after 15 minutes?

A 1, 4 and 5 B 1, 2 and 3

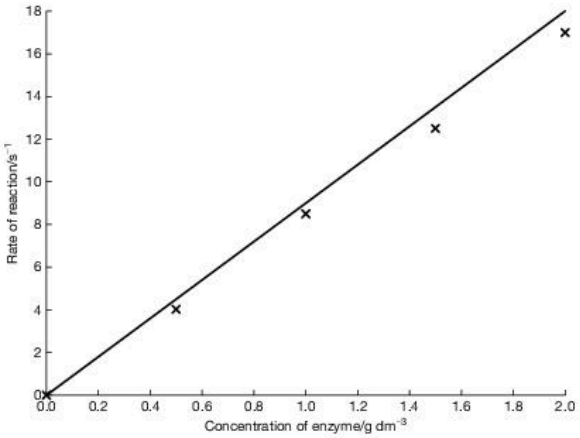
C 1, 3 and 5 D 1, 3 and 4 [1]

4 The fungus *Aspergillus niger* is the source of the extracellular enzyme β-galactosidase that is used commercially.

(a) (i) State what is meant by an extracellular enzyme. [1]

(ii) Explain why each enzyme is specific to one reaction. [3]

β-galactosidase catalyses a reaction in which the disaccharide lactose is hydrolysed into glucose and galactose. Some students investigated the effect of increasing the **concentration** of β-galactosidase on its activity. The students prepared a solution of lactose and different concentrations of β-galactosidase. The lactose solution and enzyme solutions were kept separately at pH 7 and 20 °C for five minutes before mixing them. The initial rate of each reaction mixture was determined. The results are plotted on the graph.



(b) (i) State two other variables that should be kept constant when setting up the reaction mixtures. [2]

(ii) Explain why it is important to determine the initial rate in an investigation like this. [2]

(c) Explain the results shown in the graph. [5]

(d) Predict the results that are likely to be obtained if the enzyme concentration is increased above 2.0 g dm^{-3}. Explain your answer. [2]

extra questions available online

4 Cell membranes and transport

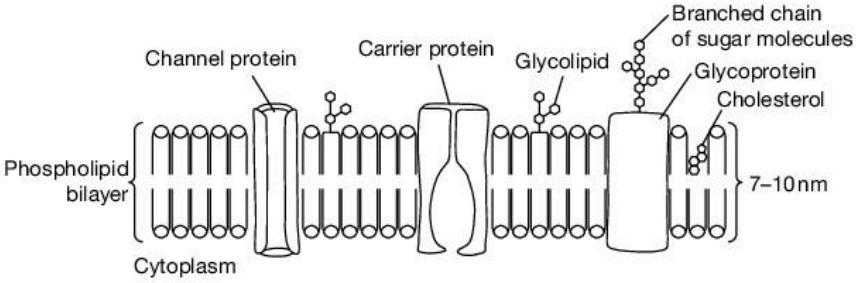
Key points

- ☐ Describe and explain the fluid mosaic model of membrane structure.
- ☐ Outline the roles of phospholipids, cholesterol, glycolipids, proteins and glycoproteins in cell membranes.
- ☐ Outline the roles of cell surface membranes and the functions of carrier proteins, channel proteins, cell surface receptors and cell surface antigens.
- ☐ Outline the process of cell signalling.
- ☐ Describe and explain the processes of diffusion, facilitated diffusion, osmosis, active transport, endocytosis and exocytosis.
- ☐ Carry out investigations on diffusion using non-living materials, such as Visking tubing and agar.
- ☐ Carry out investigations on osmosis using Visking tubing and plant tissues.
- ☐ Explain the principle of water potential.
- ☐ Calculate surface areas and volumes of simple shapes to illustrate the principle that surface area to volume ratio (SA : V) decreases with increasing size.
- ☐ Carry out an investigation using agar blocks to find the effect of changing the SA : V on diffusion.
- ☐ Carry out investigations to estimate the water potential of plant tissues by immersing them in solutions of different water potentials.
- ☐ Explain the movement of water between cells and solutions with different water potentials and explain the different effects of this movement on plant and animal cells.

The fluid mosaic membrane

A eukaryotic cell has a **cell surface membrane** (plasma membrane) that separates the contents of the cell from its surroundings. Cell surface membranes are partially permeable as they allow some substances and prevent others from moving in and out of cells. **Intracellular membranes** within cells form cell structures, such as mitochondria, endoplasmic reticulum, chloroplasts and Golgi bodies (see Unit 1).

A cell surface membrane is fluid because the phospholipids form a liquid layer in which individual molecules can move laterally (Figure 4.1). Proteins in the membranes also move, forming a scattered pattern resembling a mosaic.



▲ **Figure 4.1** The fluid mosaic structure of a cell surface membrane. The sugar chains of glycoproteins and glycolipids are only found on the outer side

Key terms

Cell surface (plasma) membrane: the partially permeable outer membrane that surrounds the cytoplasm of every cell.

Fluid mosaic model: the widely accepted idea that cell membranes are composed of a fluid phospholipid bilayer in which proteins are able to move around forming an ever changing pattern.

★ **Exam tip**

You can copy and learn this simple diagram of the cell surface membrane. You may have to draw it in answer to a question in Paper 2.

AS Level

▼ **Table 4.1** The components of cell surface membranes and their functions

Component of cell surface membranes	Function
phospholipids (see Unit 2 for structure)	• form a bilayer that acts as a barrier between cytoplasm and the surroundings of cells and is fluid so proteins can move about • hydrophilic region so can interact with aqueous cytosol and surroundings • hydrophobic region that forms the hydrophobic core of the bilayer that is permeable to non-polar molecules and impermeable to ions and large polar molecules
proteins	• transmembrane proteins form channels and carriers • enzymes that catalyse reactions on external or internal surfaces of membranes
glycoproteins – proteins with short chains of sugars (often branched) attached on exterior (outer) side	• receptors for signalling molecules, e.g. hormones and neurotransmitters • promote adhesion between similar cells to form tissues during development • cell 'markers' that are recognised by antibodies and lymphocytes (also known as cell surface antigens)
glycolipids – lipids consisting of glycerol, 2 fatty acids with one or more sugars attached on exterior side	
cholesterol	• stabilises the phospholipid bilayer by binding to polar 'heads' and non-polar 'tails' of phospholipid molecules (see page 17) • controls fluidity by preventing the phospholipid bilayer from solidifying at low temperatures and becoming too fluid at high temperatures • prevents passage of ions and polar molecules (e.g. water and glucose)

Exam tip

Diagrams of cell surface membranes are usually based on animal cell membranes. A lot of cholesterol is found in the cell surface membranes of animal cells, much less is found in plant cell membranes, but none is found in prokaryote membranes.

Remember

Proteins are made of amino acids, some of which have non-polar R-groups. The hydrophobic regions of transmembrane proteins are made of these amino acids.

Exam tip

Do not confuse cell membranes with cell walls. Membranes are made of phospholipids and proteins; cellulose is the major component of plant cell walls. Plants, fungi and prokaryotes have cell walls that surround cell membranes; animal cells do not have cell walls.

Functions of membranes at cell surfaces

▼ **Table 4.2** Some functions of cell surface membranes

Function	Comments
barrier	many water-soluble substances cannot pass across; large molecules that are required in the cell, such as proteins, cannot leave
permeability	partially permeable as some substances can pass through the phospholipid bilayer and others through transmembrane proteins; many substances cannot pass through membranes
absorption through membrane	different cell membranes are folded to different extents to increase the surface area for absorption through the phospholipid bilayer and transmembrane proteins

Function	Comments
movement by bulk flow	substances that cannot pass through the bilayer or transmembrane proteins are taken into the cell in vesicles or vacuoles formed by the cell surface membrane. When these substances are removed from a cell, they are carried to the cell surface in vesicles or vacuoles. See endocytosis and exocytosis on page 48
recognition	receptors have binding sites for cell signalling molecules such as hormones and growth factors recognising cells that must form links during tissue and organ development cell surface antigens which identify cells to the immune system

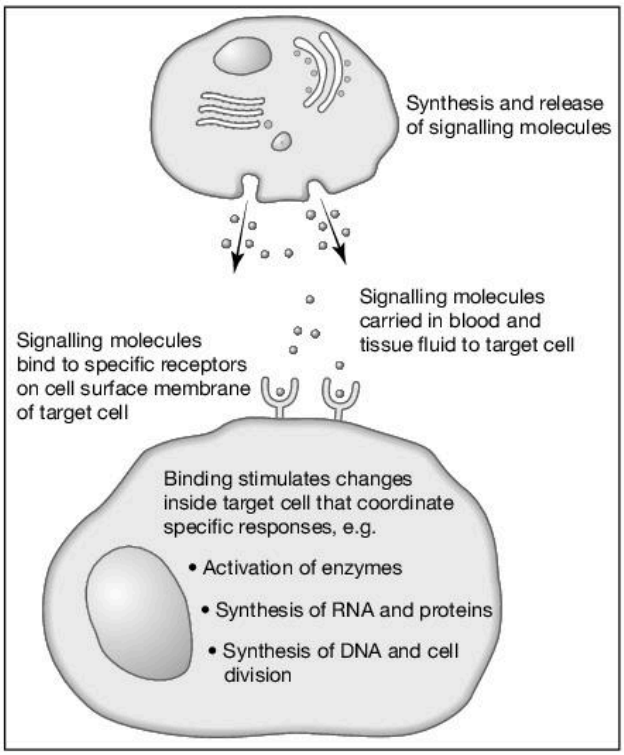
Cell signalling

Many proteins and glycoproteins on cell surfaces are receptors for chemical signals sent between cells. In animals, these chemicals can be hormones, neurotransmitters and growth factors. Some are:

- lipid soluble (e.g. the steroid hormones testosterone and oestrogen), they diffuse through the phospholipid bilayer into the cytoplasm and possibly into the nucleus, and are detected inside the cell
- water soluble (e.g. the protein hormones insulin and glucagon) and do not cross membranes. There are specific receptors on the cell surface for these two hormones and for others, such as adrenaline and many peptide hormones like antidiuretic hormone (ADH) (see Unit 14).

Only **target cells**, which have receptors for these different signalling molecules, can respond to them.

Cell signalling also occurs in plants, which have a very complex form of chemical communication involving the use of hormones, such as auxins, gibberellins and abscisic acid (ABA)(see Unit 15).



▲ **Figure 4.2** Cell signalling by a protein hormone in an animal (not drawn to scale)

The receptors on the surface of target cells bind with the signalling molecule because their shapes are complementary. The two fit together in the same way that substrate molecules fit into the active sites of enzymes.

Key term

Cell signalling: communication between cells by the release of chemicals and their detection by **target cells** that have receptors either on their cell surface or inside the cytoplasm or nucleus.

Movement across membranes

Movement across membranes is either by passive mechanisms or by active mechanisms. The latter require metabolic energy that cells must provide by making ATP in respiration.

Passive mechanisms

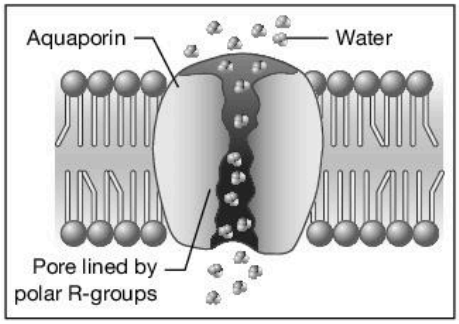
Molecules cross membranes by diffusion down their concentration gradient as a result of their own kinetic energy. The cell does not need to use metabolic energy to move the molecules.

Link

There are several units which rely on your knowledge of membrane structure, particularly protein synthesis (Unit 6), respiration (Unit 12), photosynthesis (Unit 13) and control and co-ordination (Unit 15).

Simple diffusion. Molecules pass through the phospholipid bilayer. Small, uncharged molecules, such as oxygen and carbon dioxide, pass readily through the phospholipid bilayer. Lipid (fat) soluble substances, such as steroids and fatty acids, can also pass easily by simple diffusion.

Facilitated diffusion. Unless they are very small, polar molecules cannot pass through the phospholipid bilayer so they move through channel proteins and carrier proteins. Water molecules are polar and they cannot easily pass through the bilayer. Cells have **aquaporins**, which are channel proteins specialised for the transport of water across membranes (see Figure 4.3).



▲ **Figure 4.3** An aquaporin

Channel proteins have a central space running through them lined with polar R-groups that allow ions and polar molecules to pass through the membrane. Channel proteins can remain open all the time, or open and close in response to certain stimuli.

Carrier proteins change shape (conformational change) to move ions and polar molecules, such as glucose, across membranes. In facilitated diffusion the substance concerned binds to the protein and this prompts a change in shape so that the substance is moved through the membrane. When the ion or polar molecule is deposited on the opposite side, the carrier protein returns to its original shape ready for another ion or molecule to bind.

Key terms

Channel protein: a membrane protein which has a water-filled pore through which selected ions or polar molecules can diffuse. The protein does not change shape as the ion or molecule travels through it.

Carrier protein: a membrane protein which goes through a conformational change (changes shape) to allow the movement of specific ions or polar molecules into or out of the cell by facilitated diffusion or active transport.

The factors that influence the rate of diffusion are:

- steepness of the concentration gradient between two areas, e.g. between tissue fluid that surrounds mammalian cells and cytoplasm
- surface area that the ions or molecules can diffuse through
- distance from one area to another, e.g. across a cell surface membrane of 7 nm in width
- type of ion or molecule
- size of ion or molecule
- type of cell surface membrane
- temperature.

The effect of some of these factors can be investigated with non-living materials, such as Visking tubing and agar.

Exam tip

If a substance is diffusing in one direction across a membrane, this does not affect the movement of any other substances diffusing in the same direction or in the opposite direction.

Key terms

Diffusion: movement of an ion or molecule from a place of high concentration to a place of low concentration, often described as down a concentration gradient.

Simple diffusion: type of diffusion across membranes in which substances move down their concentration gradient through the phospholipid bilayer.

Facilitated diffusion: type of diffusion in which substances move down their concentration gradient through channel proteins or carrier proteins.

Remember

The conformational changes are like the change in shape that occurs in the induced fit model of enzyme action. They are reversible changes in the tertiary structure of the protein.

Practical skills

Using Visking tubing to investigate diffusion

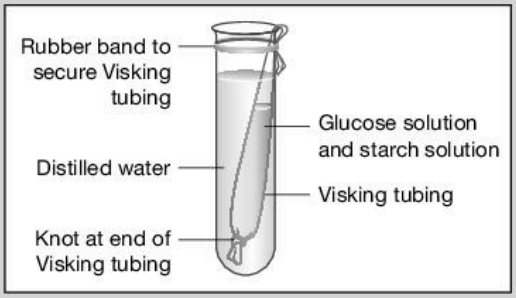
Visking tubing (dialysis tubing) is a membrane used to separate mixtures of small and large molecules. Small molecules can pass across the membrane but large molecules cannot. The tubing hydrates very quickly and is easy to open up and fill with liquids.

1 Label two large test-tubes A and B.

2 Tie a knot at the end of two lengths of soaked Visking tubing. Slide the open end of each piece between your fingers and open it out. It is best to wet your fingers first.

3 Use a syringe to add 10 cm^3 of a glucose solution and then 10 cm^3 of a starch solution to each piece of Visking tubing.

4 Rinse the outside of each piece of Visking tubing in water to remove any trace of the two solutions. Place one piece of tubing into test-tube A and the other into test-tube B. Fasten each piece to the test-tube and add distilled water (Figure 4.4).



▲ **Figure 4.4** Using Visking tubing to investigate diffusion through membranes

5 Place both test-tubes in a water bath at 40 °C for 15 minutes. Immediately take two samples of water from the test-tubes and use Benedict's solution and iodine in KI solution to test them.

6 Continue testing samples of the water from each tube every 5 minutes for 15 minutes.

Time/min	Results of tests on water surrounding Visking tubing			
	Benedict's solution		Iodine in KI solution	
	A	B	A	B
0	blue	blue	yellow	yellow
5	green	green	yellow	yellow
10	yellow	yellow	yellow	yellow
15	orange	orange	yellow	yellow

Use two pieces of Visking tubing to make sure that the results are repeatable. These results show that:

- glucose moves into the water in the test-tube because glucose molecules are small enough to diffuse through the pores in Visking tubing
- starch does not move out into the water in the test-tube through the Visking tubing because the molecules are too big, so they remain inside.

Testing a sample of the solution inside the tubing at 15 minutes gives a blue-black colour and confirms that starch is present inside the tubing.

Remember

To remind yourself about the Benedict's test for reducing sugars and the iodine test for starch, see Unit 2 page 20.

The effect of size on rates of diffusion

As a cell grows and increases in size, its surface area increases by a factor of 2, but its volume increases by a factor of 3. A large cell has less surface area per unit of volume than a small cell. As cells increase in size they do not obtain oxygen fast enough to maintain aerobic respiration. They cannot get rid of carbon dioxide by diffusion fast enough, so it accumulates in the cells and may become toxic.

Practical skills

Investigating the effect of increasing surface area to volume ratio on diffusion

Model cells made from blocks of agar can be used to investigate the effect of increasing the surface area : volume ratio (SA : V) on diffusion into cells. An alkali, such as sodium carbonate, and a pH indicator are added to molten agar and allowed to cool. A common indicator is cresol red which makes the agar purple. If the blocks are dropped into dilute hydrochloric acid, they gradually change to yellow as the acid diffuses into the blocks. The time it takes for the whole block to change colour indicates how long it would take oxygen to diffuse to the centre of a cell of that size.

Exam tip

Expect to calculate the surface areas and volumes of regular-shaped objects, e.g. cubes and cylinders.

1 Cut blocks of agar into cubes of different sizes (e.g. with sides of 20 mm, 10 mm, 5 mm, etc.).

2 Place each cube into a test-tube or small beaker and cover with dilute hydrochloric acid.

3 Time how long it takes for the acid to change the colour of the indicator in each agar block.

Alternatively, remove all the blocks from the acid as soon as the first one changes colour completely. Cut the other blocks in half and measure how far the acid has moved into the centre of each block. The results can be plotted on a graph with surface area to volume ratio (SA : V) on the *x*-axis. Note that the largest cube has the smallest SA : V and is on the left of the *x*-axis.

Exam tip

Take care when using sharp scalpels and single-edged razor blades. Always include a risk assessment in your investigation plan, e.g. in Paper 5 – see Unit 20 Practical assessment, page 244.

Osmosis

Osmosis is the passive movement of water through a partially permeable membrane from a place with a higher water potential to a place with a lower water potential. In cells that have aquaporins, most of the water that travels through membranes passes through these channel proteins, which are specialised for the movement of water (not all cells need aquaporins).

Water potential

Water potential is the tendency of water to move from one place to another and is determined by:

- the free energy of water present
- the concentration of solutes, such as ions and sugars
- the pressure exerted by the cell wall that resists any increase in volume (in plant cells and prokaryotes, not in animal cells).

Water molecules are in constant motion and therefore have kinetic energy. This free energy of water can be changed. It can be:

- *decreased* by the addition of solutes, such as sucrose and sodium chloride, because water molecules form clusters around ions and polar molecules resulting in negative water potentials
- *increased* if an external pressure is applied.

Key terms

Water potential: the free energy of a solution in comparison to pure water, which has a water potential of zero; it is a measure of the ability of a solution to absorb or lose water and is applied to cells, tissues, organs, the soil and the atmosphere.

Solute potential: the component of water potential that is due to the solutes that are dissolved in water. Solute potential is always negative.

Pressure potential: the pressure exerted by cell walls to resist the expansion of cell contents.

Pure water (with no solutes) has a water potential of zero. The addition of a solute decreases the free energy of water so that the solution formed has a negative water potential. This means that:

- solutions with low concentrations of solute molecules have negative water potentials just below zero
- solutions with high concentrations of solute molecules have much lower (more negative) water potentials
- water moves from a place with a high water potential (high free energy) to a place with a low water potential (low free energy).

Cytoplasm and the cell sap inside plant vacuoles contain many solutes, such as proteins, sugars and ions.

When cells are placed:

- in distilled water, more water molecules move into the cell down the water potential gradient than move out; the *net* movement of water is into the cell
- in solutions with high concentrations of solutes (e.g. of sucrose and sodium chloride), more water molecules move out of cells down the water potential gradient than move in; the *net* movement of water is out of the cells.

Osmosis and plant tissues

As well as carrying out the practicals in this unit on non-living materials, you are also expected to work with plant tissue. The following investigations on osmosis give results that are qualitative and quantitative.

Exam tip

You should be able to explain the results of immersing plant tissues in different solutions using the key terms defined on page 45).

The unit for measuring water potential, **solute potential** and **pressure potential** is the Pascal (Pa). Values for these are often given as kiloPascals (kPa) or MegaPascals (MPa).

Exam tip

Potato storage tissue that has been in distilled water for 12 hours cannot absorb any more water. It has a water potential of 0 kPa.

Exam tip

When writing about osmosis and the movement of water between cells and in organisms, always refer to water potential. When answering questions refer to movement of water by osmosis and water potential gradients.

Practical skills

Investigating the effect of immersing plant tissues in solutions of different water potential

Cut a vegetable such as an eggplant, *Solanum melongena*, into sections. Place the sections into sodium chloride solutions using the concentrations shown in the table and leave them for no longer than 10 minutes. Then take them out and compare each one with a freshly cut eggplant section. Observations on the eggplant tissue from each solution are shown in the table.

Concentration of sodium chloride/mol dm^{-3}	Observations on the eggplant tissue compared with freshly cut tissue
0.00 (distilled water)	firmer/harder
0.25	very similar
0.50	slightly softer
0.75	softer than in 0.50 mol dm^{-3}
1.00	very soft

The tissue that was placed in water has become harder as the cells have absorbed water and become turgid. The tissues in concentrations greater than 0.25 mol dm^{-3} have lost water and the cells have become flaccid, which is why the tissue feels softer.

▲ **Figure 4.5** Sections or cubes cut from eggplants make good material to investigate osmosis. The changes happen very quickly

A quantitative investigation using plant tissue

Choose a suitable plant tissue such as European potato, *S. tuberosum*, sweet potato, *Ipomoea batatas*, or yam, *Dioscorea alata*. The part of these plants that you eat is storage tissue.

1 Use a cork borer or a chip-making machine to cut the tissue into cylinders or 'chips'.

2 Cut all the pieces to the same length and make sure that the ends are square.

3 Weigh the pieces and record their individual masses.

4 Place the pieces into separate test-tubes containing solutions of sucrose as shown in the results table below.

5 After 12 hours reweigh the pieces and record the results.

In this investigation, it is necessary to calculate the percentage change in mass because the initial masses of the pieces are likely to be different.

The percentage change in mass is the **derived variable**.

Exam tip

As you read a procedure like this, identify the independent and dependent variables. The independent variable in this investigation is concentration of sucrose solution and the dependent variable is the change in mass.

Link

For more on variables see Unit 20 Practical assessment.

Maths skills

Percentage change

Percentage change is calculated as follows:

$$\text{percentage change} = \frac{\text{change in mass}}{\text{original mass}} \times 100.$$

Concentration of sucrose solution/ mol dm^{-3}	Initial mass/g	Final mass/g	Change in mass/g	Percentage change in mass	Mean percentage change in mass
0.0	1.26	1.49	0.23	18.3	19.3
	1.26	1.51	0.25	19.8	
	1.22	1.46	0.24	19.7	
0.2	1.22	1.31	0.09	7.4	5.4
	1.27	1.32	0.05	3.9	
	1.25	1.31	0.06	4.8	
0.4	1.43	1.29	–0.14	–9.8	–7.7
	1.32	1.26	–0.06	–4.5	
	1.37	1.25	–0.12	–8.8	
0.6	1.32	1.10	–0.22	–16.7	–15.2
	1.26	1.07	–0.19	–15.1	
	1.22	1.05	–0.17	–13.9	
0.8	1.28	0.98	–0.30	–23.4	–21.2
	1.25	1.01	–0.24	–19.2	
	1.23	0.97	–0.26	–21.1	
1.0	1.31	0.96	–0.35	–26.7	–23.3
	1.27	1.00	–0.27	–21.3	
	1.23	0.96	–0.27	–22.0	

◀ **Table 4.3** The effect of immersion in different concentrations of sucrose solution on the mass of pieces of storage tissue of European potato

Table 4.4 shows the water potentials of the six solutions of sucrose.

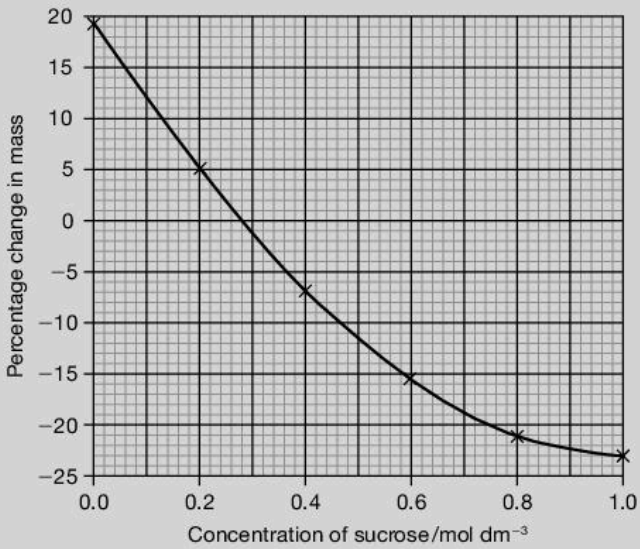
▼ **Table 4.4** The water potentials of six sucrose solutions

Concentration of sucrose solution/mol dm^{-3}	Water potential/kPa
0.00	0
0.20	−540
0.40	−1120
0.60	−1800
0.80	−2580
1.00	−3500

Three pieces of plant tissue are used for each concentration to see how much variation there is in the results. If there is little variation in the results for each concentration then you can say that the results are repeatable. The three pieces of potato cannot be labelled so they are put into separate test-tubes rather than all in the same container.

You can plot these results on a graph (Figure 4.6).

Where the curve on the graph passes through the horizontal line at 0% there is no change in mass. You can use the graph to estimate the concentration at which there is no change in mass. Here the intercept at 0% is 0.28 mol dm^{-3}. You can draw a graph of the water potentials of different sucrose solutions from Table 4.4 and use it to find the water potential in kPa of this concentration of sucrose.



▲ **Figure 4.6** The effect of immersion of potato storage tissue in different solutions of sucrose

These changes in mass are the result of movement of water by osmosis between the cells and the sucrose solution. In a solution:

- more concentrated than 0.28 mol dm^{-3}, water moves from the cells into the solution; pieces of tissue decrease in size
- less concentrated than 0.28 mol dm^{-3}, water moves from the surrounding solution into the cells; pieces of tissue increase in size
- in which there is no change in mass, there is no *net* movement of water; water molecules still move in and out of cells through cell surface membranes, which remain permeable.

★ Exam tip

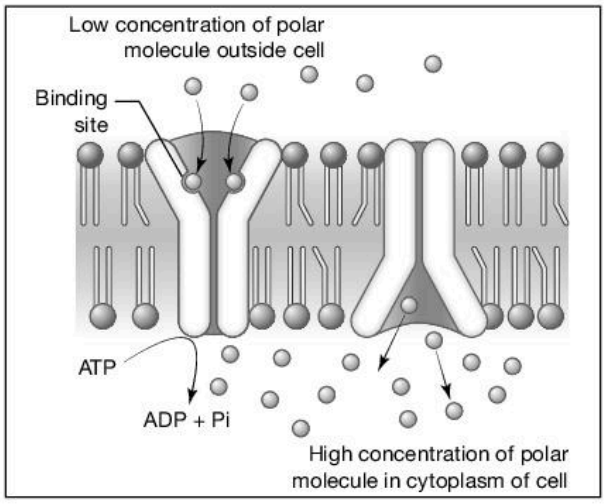
When there is no overall movement of water, it is best to write that there is no net movement of water by osmosis.

The effects of gain and loss of water on plant cells are investigated by immersing epidermal tissue from an onion in solutions with different water potentials. Cells immersed in distilled water are **fully turgid**. Water enters the cells and increases the volume of the vacuole, which pushes the cytoplasm and cell surface membrane against the cell wall. The pressure potential exerted by the cellulose cell wall withstands this turgor pressure. Cells immersed in a solution with a very low water potential are **plasmolysed**. Water diffuses out of the cells by osmosis. Most of the water comes from the vacuole that decreases in volume, pulling the cytoplasm and cell membrane away from the cell wall. The space between the cell wall and the cell surface membrane fills with the external solution. Cells can recover from plasmolysis if they receive sufficient water.

Active mechanisms

Active mechanisms require metabolic energy. There are two types: active transport and bulk flow.

Active transport. This is used to move substances required inside cells that are present in very low concentrations outside. They cannot be absorbed by passive transport mechanisms. Active transport (Figure 4.7) happens in root cells that absorb ions from soil water and in liver cells to absorb glucose from the blood.



▲ **Figure 4.7** A carrier protein using ATP to provide energy to move molecules across a cell surface membrane into the cytoplasm against a concentration gradient

Active transport is also used to move substances out of cells. Most cells have carrier proteins that move sodium ions out of their cytoplasm, which maintains a lower concentration of sodium ions inside compared with outside. This helps to reduce their water potential so they absorb less water than if the sodium ions remained in the cells.

Bulk transport. Cells take in particles or liquids from their surroundings by **endocytosis**. For example, phagocytic white blood cells take in bacteria by **phagocytosis**. Bacteria are much too large to pass through the transmembrane proteins or through the bilayer. So the bacteria are enclosed in a vacuole formed from the cell surface membrane. Each time membrane is used in endocytosis, the quantity of cell surface membrane is reduced, so more phospholipids and proteins must be added to replace it.

Cells in the pancreas make inactive enzymes that are packaged into vesicles by Golgi bodies. These vesicles travel through the cytoplasm to fuse with the cell surface membrane to release their contents by **exocytosis**.

Key terms

Phagocytosis: a type of endocytosis in which solid particles (e.g. bacteria) are taken into a cell inside a vacuole formed from cell surface membrane.

Pinocytosis: a type of endocytosis in which a liquid is taken into a cell inside a vesicle formed from cell surface membrane.

Key term

Active transport: the movement of ions or molecules by carrier proteins in cell membranes against a concentration gradient. The process requires energy supplied by ATP.

Exam tip

The carrier protein changes shape each time it moves molecules across the membrane. This change in shape is a conformational change in the protein.

Link

There is more about the carrier proteins that move sodium ions in nerve cells in Unit 15 page 173.

Key terms

Bulk transport: the movement of large quantities of particles and liquids across cell membranes by endocytosis or exocytosis.

Endocytosis: the movement of particles and liquids into a cell by the infolding of the cell surface membrane to form vacuoles or vesicles.

Exocytosis: the movement of substances out of a cell by means of vesicles that fuse with cell surface membrane to release the contents to the outside.

Worked example

(a) Explain the difference between each of the following: (i) carrier protein and channel protein; (ii) simple diffusion and facilitated diffusion across membranes; (iii) passive and active transport across membranes; (iv) signalling protein and receptor protein; (v) exocytosis and endocytosis.

(b) Plants, such as sea lavender and mangrove, live in very salty soils that have very low water potentials. Outline how you would find the water potential of the root tissue of these plants.

★ Exam tip

Part **(a)** is a comparison question requiring short answers. Part **(b)** requires an extended response with ideas written in a logical and sequential manner.

Answers

(a) (i) Carrier proteins change shape to move ions or molecules across membranes. Channel proteins do not change shape as ions or polar molecules pass through their central pore. Carrier proteins are for facilitated diffusion and active transport; channel proteins only for facilitated diffusion.

(ii) Simple diffusion occurs through the phospholipid bilayer. Facilitated diffusion occurs through channel or carrier proteins.

(iii) Active transport uses energy to drive the change in the shape of carrier proteins so that they can move ions or polar molecules into or out of a cell against the concentration gradient. Passive movement does not need any energy (ATP) from metabolism. The kinetic energy of ions and molecules is responsible for the movement by either simple diffusion or facilitated diffusion.

(iv) Signalling protein is secreted by a cell to act on a target cell to trigger a response in that cell. Receptor protein is a membrane protein with a shape complementary to a signalling molecule – binding leads to a response by the cell.

(v) Exocytosis is the movement of substances out of a cell by means of a vacuole that fuses with the cell surface membrane. Endocytosis is the movement of substances into a cell by means of a vacuole or vesicle that forms from the cell surface membrane.

(b) Follow these steps:

1 Cut pieces of root tissue all about the same size. Surface dry with pieces of paper towel.

2 Weigh the pieces and record their masses.

3 Put the pieces of root tissue into labelled test-tubes in different solutions of sucrose (e.g. those shown in Table 4.3).

4 Surface dry the pieces with paper towel after 3 hours and then weigh them again.

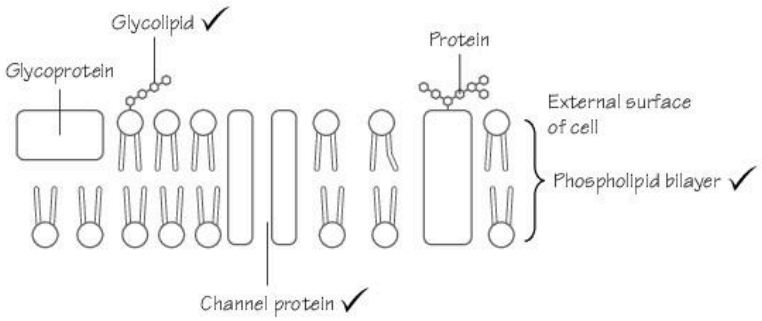
5 Calculate the percentage change in mass.

6 Plot a graph of percentage change in mass (y-axis) against the water potential of the sucrose solutions (x-axis) using the information in Table 4.4.

7 Take an intercept on the graph at 0% change and read off the water potential of the sucrose solution. This is an estimate of the water potential of the root tissue because it is predicted that there will be no net movement of water by osmosis between the tissue and a solution with this water potential.

Raise your grade

1 (a) (i) Make a simple drawing to show the structure of a cell surface membrane. [5]



The candidate has drawn a good, simple diagram showing membrane structure. Notice that one of the phospholipids has been drawn with an unsaturated fatty acid chain indicated by the kink. Some glycolipids have phosphate groups indicated by the circle, but many do not. The candidate has not drawn any cholesterol. The diagram is awarded 4 marks.

(ii) Label the components of the membrane. [5]

Labelling has not been done particularly well. A protein has been labelled as a glycoprotein even though it has no sugar molecules attached. The structure labelled as a protein is in fact a glycoprotein as it has a chain of sugars on the outer surface. The labelling is awarded 3 marks.

(b) State the width of the membrane. [1]

The membrane is 7 μm wide. ✘

Answer incorrect by a factor of a 1000! Membranes are between 6 and 10 nanometres wide, *not* 7 micrometres wide.

(c) Explain how polar molecules pass across cell surface membranes. [3]

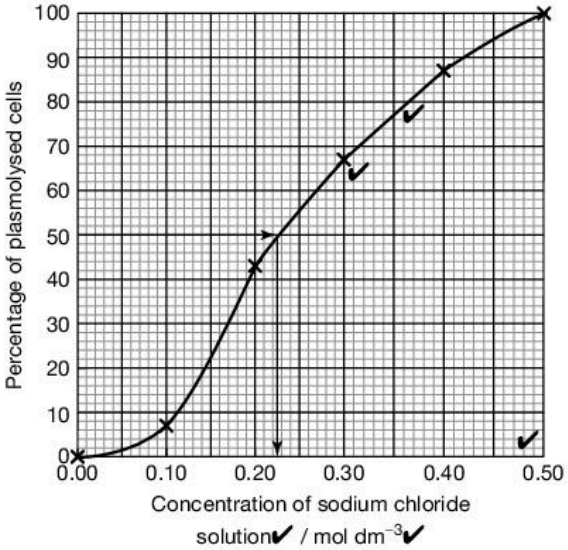
Polar molecules cannot diffuse through the phospholipid bilayer because they are hydrophilic. The molecules diffuse through proteins in the membrane as they cannot pass through phospholipids.

The first sentence did not answer the question asked, but instead answered the question 'Why do polar molecules not cross the phospholipid bilayer in membranes?' The second sentence correctly refers to proteins, but not to carrier or channel proteins, which are required.

2 Some epidermis was peeled from onion scale leaves, cut into pieces and placed in solutions of different concentrations of sodium chloride. The pieces were immersed for 10 minutes and then placed on microscope slides in the same solutions. The epidermal pieces were observed under high power. In each case, 100 cells were chosen at random and the number of these that were plasmolysed was recorded. The results are shown in the following table.

Concentration of sodium chloride solution/mol dm^{-3}	Percentage of plasmolysed cells
0.00	0
0.10	7
0.20	43
0.30	67
0.40	87
0.50	100

(a) Plot a graph of these results. [5]



Correct graph: axes are correct with the independent variable (concentration) on the *x*-axis and the dependent variable (percentage of plasmolysed cells) on the *y*-axis; both scaled correctly; *x*-axis labelled with the correct units; points plotted accurately; suitable line of best fit drawn.

(b) State the concentration of sodium chloride in which 50% of the cells should be plasmolysed. [1]

Water potential = 0.225 mol dm^{-3} ✔

The candidate has used a ruler to draw lines on the graph to show the intercept at 50% plasmolysis to find the correct answer.

(c) Explain how plant cells become plasmolysed. [4]

The water potential of the surrounding solution is lower than the water potential of the cell. ✔ Water moves out of the cell by osmosis down the water potential gradient. ✔ The cytoplasm and the vacuole decrease in size ✔ and the cell surface membrane is pulled away from the cell wall. ✔

Good answer that uses the correct terms.

Exam-style questions

1 Cell surface (plasma) membranes in mammalian cells include the following compounds.

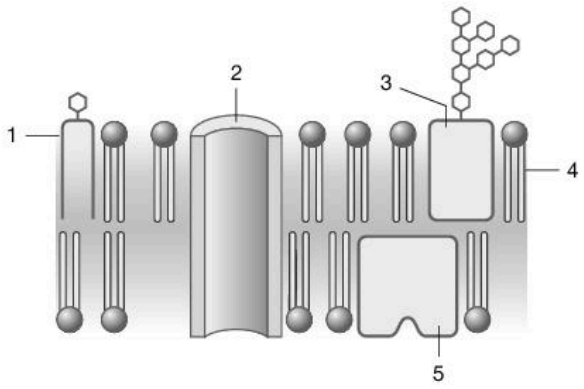
1 cholesterol, 2 glycolipids, 3 phospholipids, 4 glycoproteins

Which compound is involved in each of these functions of cell surface membranes?

	Cell recognition by lymphocytes	Allowing diffusion of non-polar substances into the cell	Detection of hormones, such as insulin	Maintaining the fluidity of membrane
A	1	4	3	2
B	2	3	4	1
C	3	1	2	4
D	4	2	1	3

[1]

2 The diagram represents a cell surface membrane.



Which of the molecules have a portion that is hydrophilic?

A 1, 2, 3, 4 and 5;

B 2, 3 and 5 only;

C 1 and 3 only;

D 4 only. [1]

3 An agar block has the following dimensions:

length = 4 mm; width = 4 mm; height = 2 mm

Which is the surface area : volume ratio for this agar block?

A 1 : 1

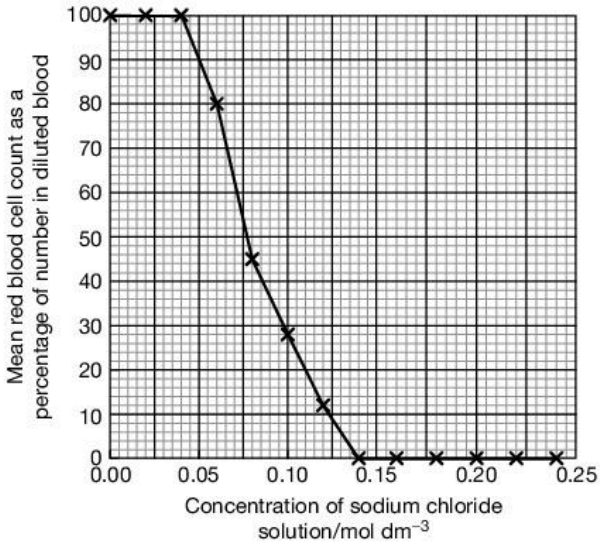
B 2 : 1

C 3 : 1

D 4 : 1 [1]

4 A student investigated the effect of immersing red blood cells into solutions of sodium chloride. The student took three samples from each solution and used a cell counter to determine the number of intact red blood cells in each sample.

The mean numbers of red blood cells for each concentration were expressed as a percentage of the cell count obtained in a sample of blood diluted with the same volume of artificial blood plasma. The percentages are shown in the graph.



(a) Explain why the student took three samples from each test-tube. [2]

(b) Use the graph to predict the concentration of sodium chloride in which 50% of the cells are destroyed by haemolysis. [1]

(c) Explain the results shown in the graph. [5]

(d) The student repeated the investigation using a culture of a single-celled alga.

State and explain how the results with this alga would differ from those shown in the graph. [4]

extra questions available online

5 The mitotic cell cycle

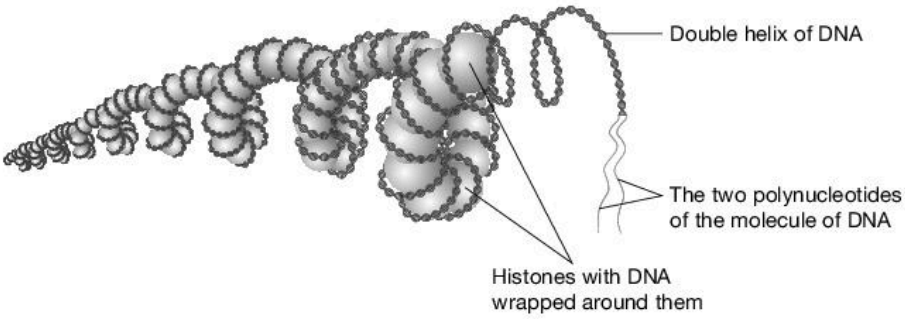
Key points

- ☐ Describe the structure of eukaryotic chromosomes, limited to DNA, histone proteins, chromatids, centromeres and telomeres.
- ☐ Explain the importance of mitosis in the production of genetically identical cells, growth, cell replacement, repair of tissues and asexual reproduction.
- ☐ Outline the mitotic cell cycle including the changes that occur during interphase, mitosis and cytokinesis.
- ☐ Outline the significance of telomeres in permitting continued replication and preventing the loss of genes.
- ☐ Outline the significance of stem cells in cell replacement and tissue repair.
- ☐ State that uncontrolled cell division can result in the formation of a tumour.
- ☐ Describe the behaviour of chromosomes in plant and animal cells during the mitotic cell cycle.
- ☐ Describe the changes that occur to the nuclear envelope, cell surface membrane and the spindle during the cell cycle.
- ☐ Observe and draw the stages of mitosis visible in root tips.

The structure of the nucleus

Eukaryotic cells have nuclei that you can see clearly with the light microscope. Some areas within nuclei stain more darkly than others. This is even more obvious in the electron microscope. The material inside nuclei is **chromatin**.

Figure 5.1 shows how the long molecule of DNA is wound around **histone** proteins to form chromosomes. In heterochromatin, the lengths of DNA and histones are much more tightly coiled than in euchromatin.



▲ **Figure 5.1** DNA is wound around histone proteins and then closely packed together to form heterochromatin

Key terms

Chromatin: the material in the nucleus composed of DNA and proteins, mainly histones. During mitosis the chromatin is seen as separate chromosomes.

Histone: type of globular protein that is found in chromosomes. Histones interact with DNA to provide a way to package a very long molecule into a very small space.

Telomere: the end of a **chromosome** which ensures that DNA molecules do not become shorter each time they are replicated.

Chromosomes

In non-dividing cells, each chromosome consists of a very long molecule of DNA. Each molecule of DNA is associated with many molecules of histones. Each histone molecule has many amino acid residues with R-groups that have amino groups ($-NH_2$). These R-groups ionise to $-NH^+$ and so interact with negatively charged phosphate groups on DNA – see Figure 6.2 on page 64. At each end of a chromosome are **telomeres**, which are segments of DNA that maintain the length of DNA molecules each time a cell divides.

Link

The telomeres have many guanine and cytosine bases. You can read more about the structure of DNA in Unit 6.

Chromosomes are visible as separate structures only during cell division when they are highly condensed with DNA and histones packed tightly together. At this time, each chromosome consists of two molecules of DNA joined together at the **centromere**. Each molecule of DNA and its associated histones is known as a **chromatid**. In dividing cells, chromosomes are first visible as separate thread-like structures when the cell begins the process of division. All the DNA is in the form of tightly packed heterochromatin which makes it possible to move the chromosomes around the cell without them becoming tangled.

During cell division it is possible to count the number of chromosomes. In body cells the number is the **diploid** number (abbreviated to 2n). In gametes, the number is the **haploid** number (n). In humans 2n = 46 and n = 23.

Key terms

Centromere: part of a chromosome where **sister chromatids** are joined together and where the chromosome is attached to the spindle during nuclear division.

Chromatid: one of the two thread-like structures joined at the **centromere** that comprise a double-stranded chromosome.

Cell division

Almost immediately after fertilisation, a mammalian zygote divides into two **genetically identical cells**. Growth by cell division continues until a hollow ball of cells is formed. This embryo is about the same size as the zygote but is now composed of many cells. Each time a cell divides, its nucleus divides first and then the cytoplasm divides to give two new cells, each with a nucleus. **Mitosis** is the nuclear division that occurs as cells increase in number like this. The nuclei that are produced are genetically identical to each other and to the parent nucleus. This maintains **genetic stability** throughout the life of an organism.

Key term

Sister chromatids: two chromatids that contain identical molecules of DNA; they are attached together at the **centromere** to form a double-stranded **chromosome**.

Later in development, the cells in the embryo gain nutrients and grow. Cells cannot grow indefinitely. When a cell reaches a certain size, it is unable to support itself as the area of the cell surface membrane is too small to absorb enough oxygen and to lose carbon dioxide fast enough to support the increase in the volume of **protoplasm** (cytoplasm plus nucleus) – the surface area : volume ratio becomes smaller. This is one reason why cells divide into two after growing for a while.

In multicellular organisms, mitosis is involved in:

- growth
- repair of tissues following wounding or other damage
- replacement of cells and tissues
- asexual reproduction
- cloning lymphocytes in the immune response.

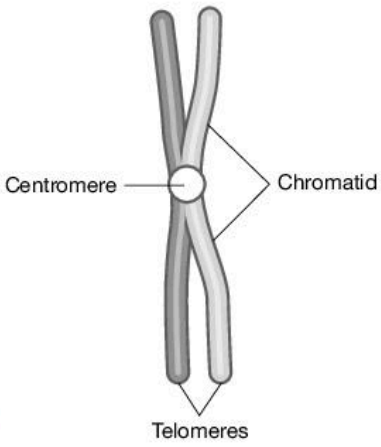
Key terms

Diploid: describes a cell or a nucleus of an organism that has two sets of chromosomes.

Haploid: describes a cell or a nucleus of an organism that has one set of unpaired chromosomes.

★ Exam tip

Numbers of chromosomes in different species vary considerably between single numbers to over a thousand. Not all species have 46 chromosomes.



▲ **Figure 5.2** A chromosome composed of two **sister chromatids** joined together at the centromere. The ends of the DNA molecules that make up each chromatid are telomeres

★ Exam tip

Do not confuse mitosis with meiosis, the other form of nuclear division. In mitosis, the chromosome number stays the same and daughter nuclei are genetically identical. In meiosis, daughter nuclei have half the chromosome number of the parent cell and are genetically different.

In multicellular organisms, cells produced by cell division often remain together to form tissues and differentiate to become specialised to carry out specific functions.

Other undifferentiated cells can continue dividing by mitosis to produce more and more cells, which can specialise.

In animals these are **stem cells**, which divide:

- to form new cells that specialise to form new tissues, to replace worn out tissues or to repair damaged tissues with replacement cells
- in the bone marrow – to replace red and white blood cells that have a limited life span
- at the base of the epidermis in the skin – to replace the cells at the surface that are constantly rubbed off, and to form cells to cover wounds and replace the damaged cells when the skin is cut.

In plants, these are **meristematic cells**, which are found in areas called **meristems**; for example, root tips (page 58), shoot tips, and the cambium, which gives rise to xylem and phloem tissues (see Unit 7 pages 74–77).

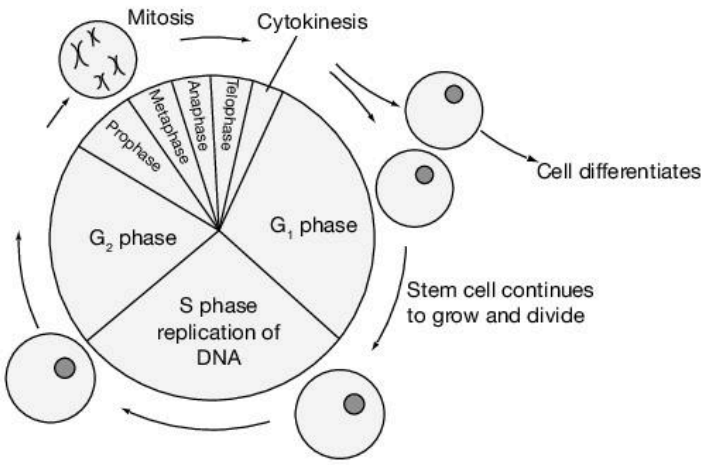
Mitosis is also involved in asexual reproduction of multicellular organisms. This happens in fungi, in some animals and in many plant species.

The cell cycle

During the **mitotic cell cycle** the nucleus divides first to form two daughter nuclei, usually followed by the division of the cytoplasm to give two daughter cells, each with its own nucleus. Sometimes nuclear division is not followed by cytoplasmic division as happens in many fungi that have hyphae (long thin threads) that are not subdivided into cells.

Key term

Mitotic cell cycle: all the changes that occur within a cell between its formation and its division by **mitosis** and **cytokinesis** into two cells.



▲ **Figure 5.4** The mitotic cell cycle. The proportion of time a cell spends in mitosis is very short. The longest stage is interphase, which comprises the G_1 phase, the S phase and the G_2 phase

Link

For more about meiosis, see Unit 16.

Remember

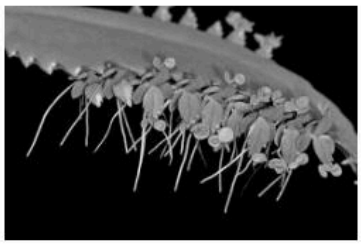
A **tissue** is a group of cells (one type or a few types) that work together to carry out the same function or functions.

Exam tip

A common error is to write 'repair of cells' as a function of mitosis. A cell cannot be repaired by dividing into two. It can, however, be destroyed and *replaced* by cells formed by the division of **stem cells**.

Key term

Cytokinesis: division of cytoplasm into two, following nuclear division (mitosis or meiosis).



▲ **Figure 5.3** These plantlets are formed from meristematic cells that divide at the edge of the leaves of the Mexican hat plant, *Bryophyllum daigremontianum*. This is an example of vegetative (asexual) reproduction

After a new cell is formed, the following events occur in this sequence:

- **growth** of cytoplasm (G_1) – molecules such as nucleoside triphosphates (activated nucleotides) are made in preparation for replication and protein synthesis (transcription and translation); amino acids are synthesised and attached to tRNA ready for translation (see Unit 6)
- **synthesis** phase (S) – DNA replication
- **growth** of cytoplasm (G_2) – organelles such as chloroplasts and mitochondria divide, more membrane is formed and polysaccharides and triglycerides are stored for energy
- mitosis (division of the nucleus)
- cytokinesis (division of the whole cell).

Replication almost always results in identical DNA, so the genetic information inherited by the two daughter cells is identical to that of the parent cell. If replication is not like this, then the cells may differ genetically and not function together in a tissue. When a genetically different cell begins to express different proteins on its cell surface, the immune system eliminates it. If it does not, the cell may divide and grow to form a tumour.

Exam tip

You can recall the sequence of stages of mitosis by the first letter of each phase – PMAT. Remember that interphase is not a stage of mitosis, but it is a stage of the cell cycle so IPMAT is correct for learning the whole cell cycle.

Key terms

DNA replication: the process in which an exact copy of DNA is made.

Tumour: a mass of cells produced by uncontrolled cell division.

Telomeres

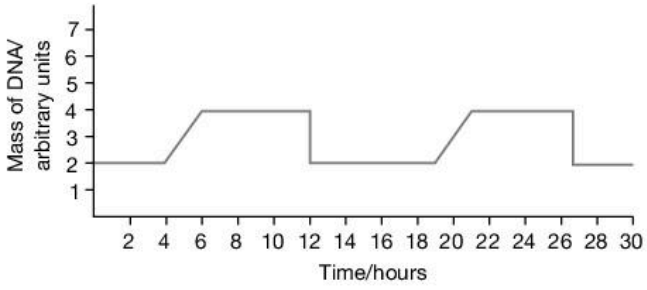
Telomeres prevent the loss of DNA from the ends of chromosomes and thus prevent the loss of genes. Each telomere consists of a sequence of nucleotides repeated many times. These nucleotide sequences do not code for any proteins. The enzyme that forms new DNA during replication (DNA polymerase) cannot continue right to the very end of a molecule of DNA. Another enzyme, telomerase, can use these repeated sequences to copy enough DNA to complete replication so that DNA molecules do not shorten during every cell cycle. If telomeres are not copied, the cell is unlikely to complete the cell cycle and will be destroyed.

Exam tip

DNA replication, the synthesis of biological molecules and the formation of membranes and organelles that all occur during interphase are highly active processes. Therefore, do not call interphase a 'resting stage'.

Worked example

The DNA content of cells changes throughout the cell cycle. This graph shows the changes that occur.



▲ **Figure 5.5** Changes in DNA content in a cell during a mitotic cell cycle

a) Outline what happens at each stage of the cell cycle to bring about the changes in the quantity of DNA shown in the graph.

b) Explain briefly how mutations of the genes that control the cell cycle lead to the formation of tumours.

c) The enzyme that catalyses the formation of DNA during replication is unable to copy the ends of each chromosome. Outline how cells avoid the progressive loss of genes from the ends of chromosomes with every cell cycle.

Answers

a) The quantity of DNA increases during the S phase (4–6 hours) because this is when DNA replication occurs so that each chromosome has two molecules of DNA. After the S phase there is twice as much DNA as in the G_1 phase. During cytokinesis (12 hours) the mass of DNA halves as the cell divides into two and each cell gets a complete set of single-stranded chromosomes from the original parent cell. Each chromosome has one DNA molecule. Between 12 and 19 hours the quantity remains constant in G_1 of the next cycle as no replication occurs in this stage.

b) The cell cycle is not controlled properly. Cells with these mutations divide uncontrollably to produce many cells that form a tumour. These cells do not respond to signals that tell normal cells not to divide.

c) There are telomeres at the ends of each chromosome. Each telomere is made of a short sequence of nucleotides that is repeated many times. Telomeres are copied by another enzyme, telomerase, so that they can be synthesised during replication. This makes sure chromosomes do not become shorter during each S phase of the cell cycle and prevents the loss of genes that would otherwise happen.

Mitosis ensures that each new cell has exactly the same genetic information as its parent cell. All the cells in the body (except for gametes) have identical genetic information so that they can all work together efficiently.

As a result of mitosis:

- two daughter nuclei are produced
- the two daughter nuclei have the same number of chromosomes as each other and the same as the parent nucleus
- genetic stability is maintained throughout the life of an individual because the genetic information is the same in the daughter cells as in the parent cell
- there is no genetic variation between cells.

Remember

Remember that prokaryotes do not have a nucleus or linear chromosomes like those of eukaryotes. Prokaryotes do not divide by mitosis.

Link

The pathogens that cause cholera and TB are bacteria – see Unit 10.

Practical skills

Observing the mitotic cell cycle

DNA is replicated during the cell cycle and this is followed by mitosis. You can see the stages of mitosis for yourself if you make a temporary preparation of the cells from the meristem of a plant, such as the root tip of onion or garlic. You can use the following procedure with cloves of garlic, *Allium sativum*.

1 Score the underside of a clove of garlic with a sharp knife and suspend the garlic over water so the base just touches the water surface.

2 After a day, remove some intact roots.

3 Cut off 10–20 mm of the root tips. Put in a small volume of ethanoic acid on a watch glass (or other shallow dish) for 10 minutes.

4 Heat 10–25 cm^3 of 1 mol dm^{-3} hydrochloric acid to 60 °C in a water bath. (The acid hydrolyses the middle lamella that holds plant cells together.)

5 Wash the root tips in cold water for 4–5 minutes and dry on filter paper.

6 Use a mounted needle to transfer the root tips to the hot hydrochloric acid and leave for 5 minutes.

7 Wash the root tips again in cold water for 4–5 minutes and dry on filter paper.

8 Use the mounted needle to remove two root tips onto a clean microscope slide.

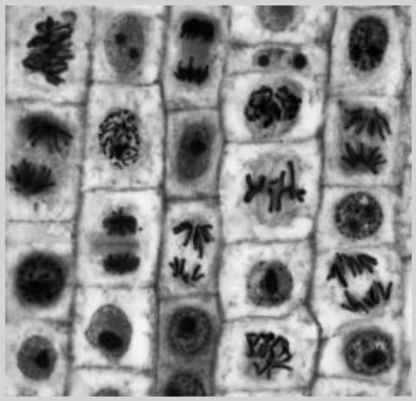
9 Use a scalpel to remove all the tissue except for 2 mm from the growing root tip. Discard the rest, but keep the tips.

10 Add a small drop of ethano-orcein (acetic-orcein) stain or toluidine blue stain and leave for 2 minutes.

11 Break up the tissue with a mounted needle.

12 Place a cover slip over the root tips. Place filter paper over the cover slip and press gently to spread the cells. Alternatively tap the surface of the cover slip gently with the blunt end of a mounted needle or pencil.

13 Use the low power of the microscope to search the slide for cells like those in the photomicrograph.



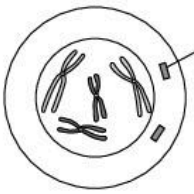
▲ **Figure 5.6** A stained section of a root tip meristem of garlic. There are cells here in various stages of the cell cycle. Use Figure 5.7 to help you find cells from each stage

Exam tip

You may be asked to calculate magnification from a slide or drawing. If you are unsure about using significant figures in calculations, see Unit 20 Practical assessment page 237.

Link

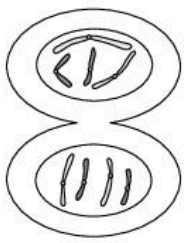
For more about making drawings from slides and drawing technique, see Unit 20 Practical assessment page 242.



Prophase
Chromatin condenses and becomes tightly packaged. The chromosomes shorten and thicken and become visible with two sister chromatids attached to each other at a centromere. The centrioles move to the poles at opposite ends of the cell and begin to produce microtubules to form the **spindle apparatus**. The nuclear envelope breaks up into small pieces that disperse throughout the cytoplasm.

Metaphase
The spindle is fully formed with microtubules attached to centromeres. The chromosomes are arranged across the equator of the cell. In this stage it is easiest to see individual chromosomes and to take images of them. Some microtubules are not attached to centromeres but extend from pole to pole.

Anaphase
The centromeres divide and the sister chromatids are pulled to opposite poles with the centromeres leading. Spindle microtubules are anchored at the poles so when they are broken down they shorten pulling the sister chromatids apart. Once the sister chromatids have separated they are now single-stranded chromosomes, each composed of one molecule of DNA.



Telophase
Chromosomes arrive at the poles and uncoil. They can no longer be seen as separate structures. The nucleolus reappears. The nuclear envelope reforms from pieces of rough endoplasmic reticulum. Euchromatin reappears within each nucleus. Nuclear division by mitosis has ended.

Cytokinesis
During or after telophase, the cytoplasm divides. A ring of microtubules forms below the cell membrane which contract to draw in the membrane to form a cleavage furrow. The membrane fuses, separating the two new cells.

Exam tip

Microtubules are made of globular protein molecules joined together to make hollow tubes. They form part of the cytoskeleton and can quickly be assembled and broken down, as in mitosis.

▲ **Figure 5.7** The stages of mitosis in an animal cell

Mitosis in plant cells

When you look at cells in different stages of mitosis in plant tissue, note that they do not have centrioles or centrosomes. Instead the microtubules are arranged into the **spindle apparatus** by an area known as the microtubule-organising centre (MTOC). As plant cells have cell walls, cells cannot divide into two by forming a cleavage furrow as in animal cells. A cell plate made of cellulose and other cell wall materials forms between the two nuclei during telophase. Cell surface membrane forms on either side of the cell plate to divide the cell into two and the new cells make more cell wall material.

Key term

Spindle apparatus: **microtubules** assemble to form fibres that extend from the poles to the **centromeres** and move chromosomes apart during **anaphase** of nuclear division.

Worked example

A student made a series of sections through the root tip meristem of an onion, *Allium cepa*. The student counted the number of cells in each stage of the cell cycle and presented the results in a table. The student discovered that it takes 13 hours at 25 °C to complete the cell cycle.

Stage of cell cycle	Number of cells	Percentage of cells in each stage	Length of time of each stage/min
interphase	254	73.6	574
prophase	45		
metaphase	16	4.6	36
anaphase	7	2.0	16
telophase	23		
total	345	100.0	780

a) Complete the table by (i) calculating the number of cells in prophase and telophase as a percentage of the total number counted, and (ii) calculating the length of time of each of these stages.

b) Suggest the conclusions that the student could make about the relative length of time of each stage in the cell cycle.

c) Describe what happens to a root tip cell during and immediately after telophase.

★ Exam tip

The percentages are calculated by dividing the number of cells in each stage by the total number of cells counted and multiplying the answer by 100.

Answers

a) (i) and (ii) Prophase: 13.0% and 102 min. Telophase 6.7% and 52 min.

b) The longest phase is interphase, which took over 70% of the time. Cell growth, DNA replication and protein synthesis occur during interphase. This stage is also when energy is stored for mitosis and cytokinesis and enough new organelles and biochemicals are produced to share between the two daughter cells. The next longest stage is prophase when chromosomes coil up and the spindle apparatus is formed. The shortest phase is anaphase when chromosomes move to opposite poles. This movement is quite fast so the stage does not take very long.

c) The nuclear envelope reforms to surround the chromosomes that are uncoiling to form chromatin. A cell plate forms between the two daughter nuclei. New cell surface membrane forms on either side of the cell plate. Once the two cell surface membranes are formed the parent cell is divided into two. Cell walls are formed on either side of the cell plate and the cell has now divided into two daughter cells.

Raise your grade

1 (a) Outline the processes that occur to prepare a cell for division and explain why these processes need to occur. [5]

It is important that more DNA is produced by semi-conservative replication.✔ Each molecule of DNA in every chromosome is copied so there are two copies for when the cell divides into two. It is also important that the cell grows and makes new organelles.✔ The new cells need enough mitochondria to make ATP so more of them need to be made.✔ More membrane also needs to be made so there is enough for the two new cells when they divide in cytokinesis.✔

The second sentence is too vague to gain a mark for any detail about replication or chromatids. Instead of writing 'two copies of DNA', it is better to refer to the polynucleotides as in Unit 6 page 63.

(b) Name the stage of mitosis in which each of the following occurs:

(i) sister chromatids are separated when centromeres split apart; Anaphase✔

(ii) chromosomes condense; Prophase✔

(iii) sister chromatids move to opposite poles; Telophase✘

(iv) nuclear envelope reforms; Interphase✘

(v) chromosomes assemble at the equator of the cell. Metaphase✔ [5]

Telophase is incorrect for **(iii)**, sister chromatids move to opposite poles during *anaphase*. However, telophase is the correct response to **(iv)**, *not* interphase.

(c) Explain the role of mitosis in the growth of animals and plants. [3]

The daughter cells produced by mitosis are genetically identical.✔ This means that they all have the same DNA and identical genes and all the cells in an animal or a plant can work together. If any of the cells were genetically different they might be attacked by other cells (e.g. by the immune system in animals)✔ or divide uncontrollably to form tumours which could harm the organism.

It is better to say that the daughter cells have identical alleles of all the genes in the organism. This is because all individuals of the same species have the same genes. If you need to be reminded about genes and alleles see Unit 16.

(d) State **three** roles of mitosis in animals and plants other than growth. [3]

Asexual reproduction,✔ cell replacement✔ and repair of tissues.✔

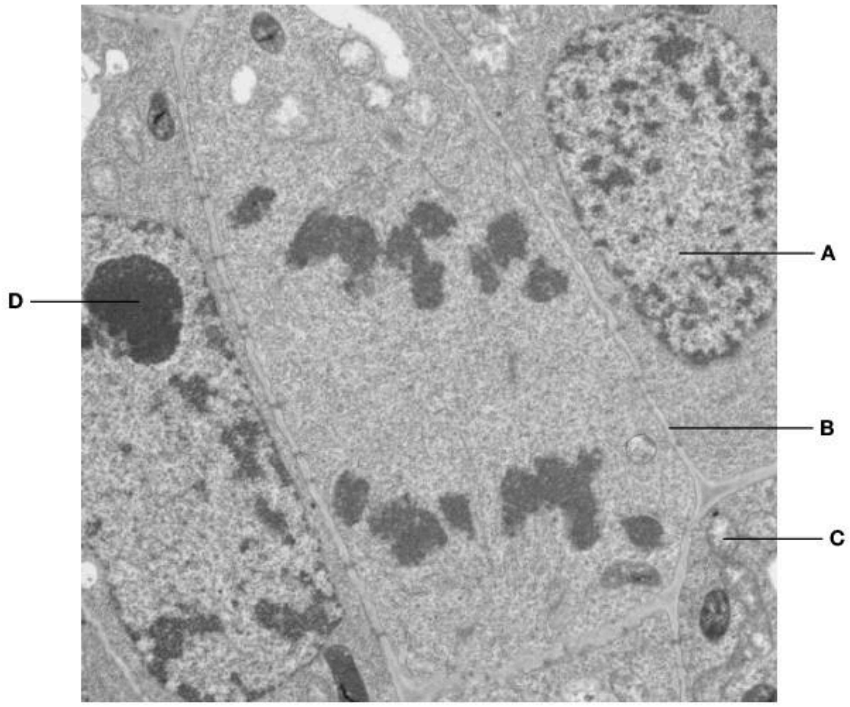
Correct answers.

(e) Suggest what might happen to the daughter cells at the end of one mitotic cell cycle. [2]

Both daughter cells may grow and divide again.✔ Both cells may change into specialised cells and so do not divide again.✔

Good answer. The candidate has noticed that there are 2 marks for this question and has given two different suggestions. Another suggestion is to use Figure 5.4 and say that one cell remains to divide and the other becomes specialised and stops dividing.

2 The photograph is a transmission electron micrograph (TEM) of a cell from the shoot tip meristem of a plant.



(a) (i) Name the structures labelled **A**, **B**, **C** and **D**. [4]

A – nucleus ✔ B – cell wall ✔ C – vacuole ✘ D – nucleolus ✔

C is a mitochondrion not a vacuole. Plant mitochondria are more difficult to identify than those in animal cells, but look carefully and you can see the infoldings (cristae) of the inner membrane.

(ii) The cell in the TEM is in the process of dividing by mitosis.
State the stage of mitosis and explain your answer. [3]

The stage of mitosis is anaphase. ✔ The EM has no nucleus so cannot be in prophase or telophase. ✔ The darkly stained areas are parts of chromosomes that appear to be moving towards the ends of the cell. ✔

Good answer, although the candidate refers to 'ends' rather than poles. The reasoning is good although the cell could be in metaphase but without more sections through the cell it is impossible to be certain, so the examiners would accept either metaphase or anaphase.

(b) Explain the advantages of using a light microscope to study the behaviour of chromosomes rather than the electron microscope. [3]

It is possible to view all of the chromosomes, ✔ not just a very thin section as in a TEM. In a thin section, you cannot see all the chromosomes. Also you can see living processes ✔ in the LM which is not possible in the EM, so you could take a video of mitosis or set a camera to take photos every 5 minutes or so (time-lapse).

The candidate should have said that taking time-lapse photos or a video makes it possible to view the *movement* of the chromosomes.

Exam-style questions

1 In root tip cells the formation of the cell plate that begins the separation of daughter cells occurs. In which stage of the cell cycle do daughter cells separate from each other?

A anaphase;

B cytokinesis;

C interphase;

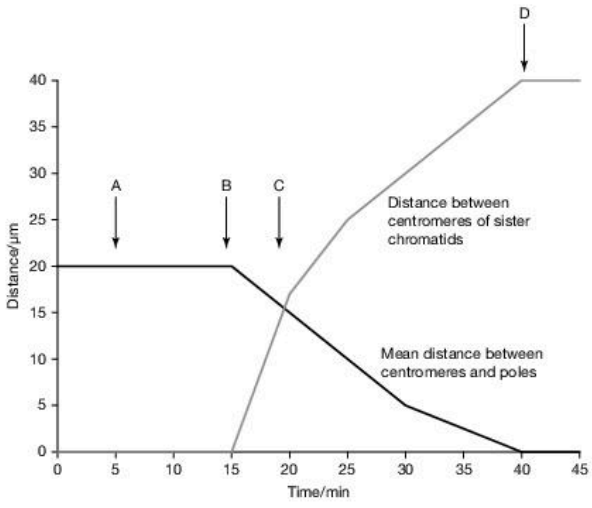
D prophase [1]

2 Which row shows the stages of mitosis when DNA is replicated, the nuclear envelope forms and sister chromatids separate?

	DNA replication	Formation of nuclear envelope	Separation of sister chromatids
A	anaphase	interphase	prophase
B	cytokinesis	metaphase	telophase
C	interphase	telophase	anaphase
D	prophase	cytokinesis	metaphase

[1]

3 The graph shows the distance between the centromeres of sister chromatids and the distance between centromeres and the poles during mitosis.



Which shows the beginning of anaphase? [1]

4 Which shows the role of telomeres?

A stimulates the decondensing of chromosomes during telophase

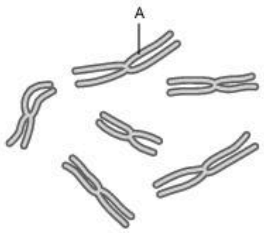
B attaches chromatids to the spindle at metaphase

C prevents shortening of DNA at the ends of chromosomes

D provides support for the DNA molecule in each chromosome [1]

5 The yellow fever mosquito, *Aedes aegypti*, has a diploid number of 6.

The drawing is made from a photograph of the chromosomes taken at metaphase of mitosis.



(a) The length of chromosome A is 8.4 μm. Calculate the magnification of the drawing:

- write out the formula that you will use
- use the formula to calculate the magnification
- give your answer to two significant figures. [3]

(b) (i) Draw a diagram to show the arrangement of any two of the chromosomes of *Aedes aegypti* in the middle of anaphase of mitosis. [3]

(ii) On your diagram label a centromere and indicate the position of the poles of the spindle apparatus. [2]

(c) Describe what happens to the nuclear envelope and the cell surface membrane during the cell division of a cell of an animal, such as *A. aegypti*. [4]

(d) At either end of each chromosome shown in the drawing are telomeres.

Outline the role of telomeres. [3]

6 Nucleic acids and protein synthesis

Key points

- ☐ Describe the structure of nucleotides (including ATP), DNA and RNA.
- ☐ Explain the importance of base pairing and hydrogen bonding in DNA.
- ☐ Describe the semi-conservative replication of DNA.
- ☐ State that a polypeptide is coded for by a gene, and that a gene is a sequence of nucleotides that forms part of a DNA molecule.
- ☐ Describe how a nucleotide sequence codes for the amino acid sequence in a polypeptide.
- ☐ Describe how the information in DNA is used during transcription and translation to construct polypeptides.
- ☐ Describe the roles of messenger RNA (mRNA), transfer RNA (tRNA) and ribosomes in the synthesis of protein during transcription and translation.
- ☐ State that a gene mutation is a change in the sequence of nucleotides that may result in an altered polypeptide.
- ☐ Explain how the nucleotide sequence of the alleles Hb^A (normal) and Hb^S (sickle cell) of the gene for β-globin code for slightly different polypeptides.

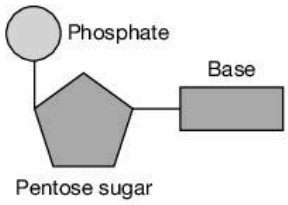
Polynucleotides

There are two nucleic acids:

- deoxyribonucleic acid (DNA)
- ribonucleic acid (RNA)

These polymers are built up from **nucleotide** monomers (Figure 6.1). DNA is a large, stable molecule that is a long-term store of genetic information. Three types of RNA use the information from DNA in the synthesis of polypeptides:

- messenger RNA (mRNA)
- ribosomal RNA (rRNA)
- transfer RNA (tRNA).



There are five different organic bases: **pyrimidine** bases have one ring composed of carbon and nitrogen atoms and the **purine** bases have two rings. Nucleotides join together to make polynucleotides by forming **phosphodiester bonds** between the phosphate group of one nucleotide and the pentose sugar of the next.

▼ **Table 6.1** A summary of the nitrogen-containing bases in DNA and RNA

	Nitrogen-containing bases				
	Purines		Pyrimidines		
Number of rings	2		1		
Bases	adenine (A)	guanine (G)	cytosine (C)	thymine (T)	uracil (U)
DNA	✔	✔	✔	✔	✘
RNA	✔	✔	✔	✘	✔

Key term

Nucleotide: monomer of the nucleic acids, **RNA** and **DNA**. Each nucleotide is composed of a pentose sugar, a phosphate group and a nitrogenous base.

◀ **Figure 6.1** A simple diagram of a nucleotide. Different shapes are used to show the five different bases found in nucleic acids, DNA and RNA

★ Exam tip

Do not confuse the structure of DNA with the structure of proteins. The monomers of DNA are nucleotides; the monomers of proteins are amino acids.

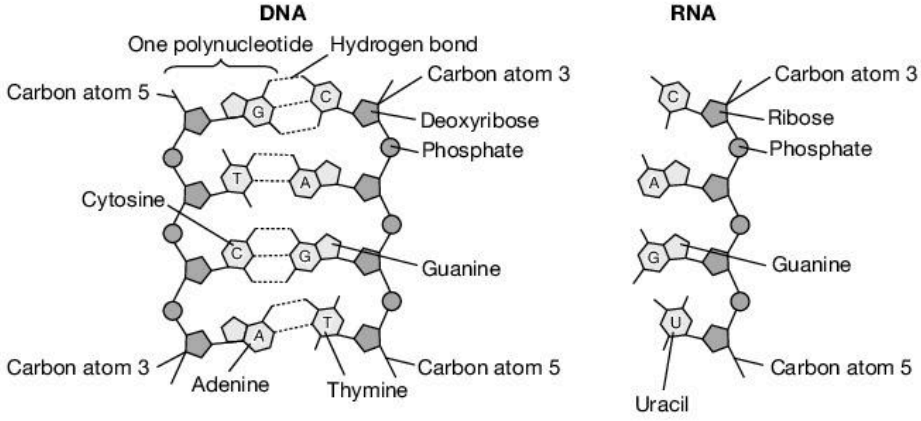
★ Exam tip

In RNA 'U replaces T'. Remember this as you work through the next few pages. DNA has thymine, RNA has uracil instead.

AS Level

DNA and RNA

A molecule of DNA is a double helix consisting of two polynucleotides (double-stranded) that are held together by **hydrogen bonds**. The deoxyribose sugars and phosphates make up the sugar–phosphate 'backbone' of each polynucleotide with the bases projecting inwards and forming hydrogen bonds with the bases of the other polynucleotide. DNA is double-stranded in eukaryotes and prokaryotes (see Figure 6.2). Some viruses have single-stranded DNA. mRNA is a single-stranded molecule (Figure 6.2). tRNA and rRNA are mostly single-stranded, and have some regions with **base pairing**.



▲ **Figure 6.2** The structure of small sections of DNA and RNA

Exam tip

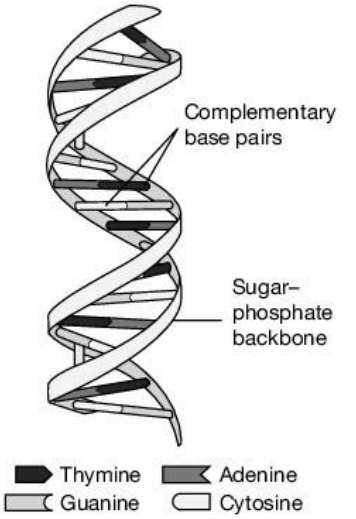
You can expect to be asked to compare DNA and RNA and also to discuss the significance of the differences between them.

Remember

Look back to Unit 2 page 11 for information about hydrogen bonds.

Link

The structure of viruses is described in Unit 1 page 1.



▲ **Figure 6.3** The double helix structure of DNA

▼ **Table 6.2** The features of DNA compared with RNA

Feature	DNA	RNA
Polynucleotides	2	1
Pentose sugar	deoxyribose	ribose
Nucleotide bases	adenine (A), guanine (G), cytosine (C) and thymine (T)	adenine (A), guanine (G), cytosine (C) and uracil (U)
Types	1	3: messenger (m) RNA, transfer (t) RNA and ribosomal (r) RNA
Base pairing by hydrogen bonding	between polynucleotides	mRNA – none tRNA and rRNA have base pairing in some regions
Ratio of base pairs	A:T = 1:1; C:G = 1:1 A+G:C+T = 1:1 (because of base pairing) A+T:C+G is constant in each species, but varies between species (as different species have different genes)	A:G ≠ 1:1; C:U ≠ 1:1 A+G:C+U varies (as there is no base pairing)
Stability	stable	less stable than DNA; mRNA less stable than tRNA and rRNA
Function	storage of genetic information	mRNA – transfers genetic information from DNA to ribosomes tRNA – identifies and carries amino acids to ribosomes rRNA – structure of ribosomes; catalyses formation of peptide bonds

ATP is a phosphorylated nucleotide

Adenosine triphosphate (ATP) is a phosphorylated nucleotide, which means it has a structure like that shown in Figure 6.1 with the addition of two more phosphate groups. The base is adenine and the pentose sugar is ribose (see Figure 12.1 page 132 for a diagram of ATP).

Exam tip

ATP is the main molecule used in energy transfer in all organisms. It is also used as one of the monomers to form RNA.

Worked example

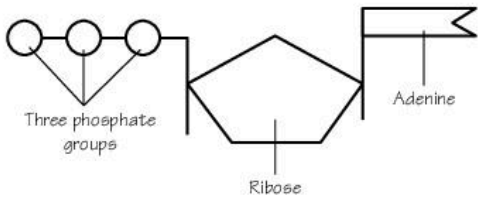
(a) (i) ATP is described as a phosphorylated nucleotide.

Make a simple labelled diagram of ATP.

(ii) State how ATP differs from the nucleotides in a molecule of DNA.

Answers

(a) (i)



(ii) ATP has ribose and three phosphate groups whereas a nucleotide in DNA has deoxyribose and one phosphate group.

DNA replication

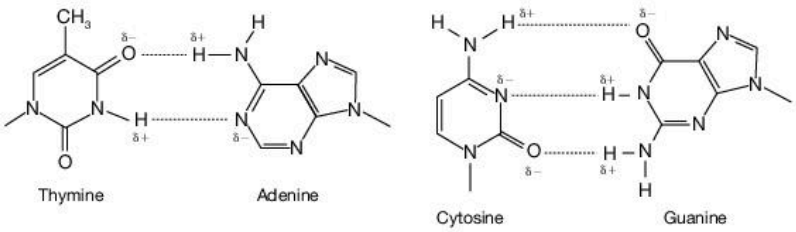
Templates and base pairing

DNA is a store of genetic information and is passed on to new cells in growth and to new generations of organisms in asexual and sexual reproduction.

The double polynucleotide structure is ideal for replication because each polynucleotide acts as a template for making a new one. This is called **semi-conservative replication**. The term template means that a copy is made by pairing nucleotide bases against an already existing strand. Each polynucleotide has a sequence of bases and within DNA you know that adenine always pairs with thymine and cytosine always pairs with guanine. Nucleotides with these bases are assembled one at a time along an existing polynucleotide to give a complementary sequence of bases. The newly assembled nucleotides are joined together by phosphodiester bonds and the bases form hydrogen bonds with the template polynucleotide. There are two hydrogen bonds between base pair A:T and three between base pair C:G (Figure 6.4). Each hydrogen bond is weak, but collectively they provide stability for each molecule of DNA because the two strands are not easily separated.

Key term

Semi-conservative replication: each **polynucleotide** in DNA acts as a **template** for the assembly of nucleotides. Each new molecule of DNA consists of a newly synthesised polynucleotide and the template polynucleotide from the original molecule.



▲ **Figure 6.4** There are two hydrogen bonds between adenine (A) and thymine (T) and three between cytosine (C) and guanine (G) in DNA

Before replication can occur, nucleotides need to be synthesised. This happens in the cytoplasm and uses energy to form activated nucleotides that have three phosphate groups not one.

Replication ensures that the sequence of base pairs always remains the same, although occasionally mistakes occur. These mistakes are mutations (see page 70).

Link

You can follow animations of replication at www.johnkyrk.com and at www.dnaftb.org/20.

Protein synthesis

Overview of protein synthesis

Protein synthesis involves the following processes:

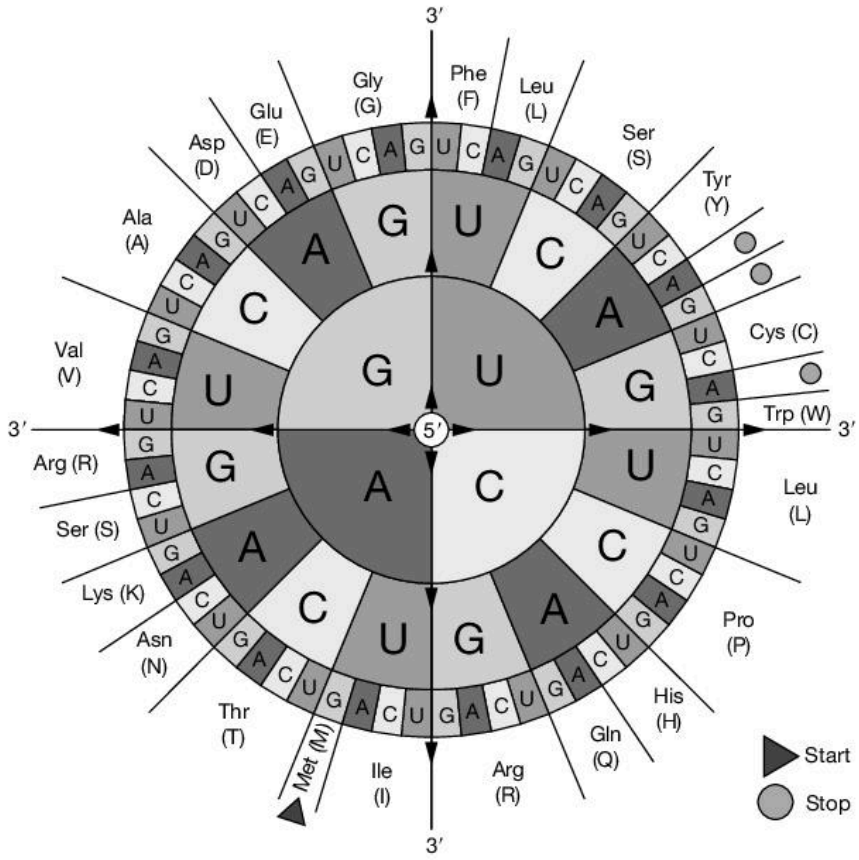
- **transcription** of DNA in the nucleus to produce mRNA
- activation of amino acids which involves attaching them to tRNA molecules – this occurs in the cytoplasm
- **translation** of mRNA on ribosomes to form polypeptides
- post-translational modification inside the RER and in the Golgi body.

Genetic code

DNA is a store of genetic information for the synthesis of polypeptides. The sequence of bases in a length of DNA codes for the assembly of amino acids to make polypeptides. Each triplet of bases is a DNA **codon**. On mRNA each triplet is an RNA codon. There are four bases in DNA (A, T, C and G) and four in RNA (A, U, C and G) so it is possible to make 64 different codons.

There are codons for each of the 20 amino acids. Methione (Met) has only one codon (AUG), while most have two or more (see Figure 6.5). There are three triplets that do not code for any amino acid; these codons are 'stop' codons that indicate the end of a sequence of codons for a polypeptide.

The sequences of the bases in the two DNA polynucleotides relate to the sequence in mRNA (Figure 6.6). The polynucleotide along which the sequence of RNA nucleotides is assembled is the **template strand** for transcription; the complementary polynucleotide is the non-template (coding) strand. The non-template strand has a sequence of bases, which is the same as that of the mRNA produced except that U replaces T. A single sequence of bases for a gene, like that in Figure 6.6, shows you the non-template strand.



◀ **Figure 6.5** The mRNA wheel. This shows the genetic code in the form of mRNA codons (e.g. GGG for glycine (Gly)). The amino acids are identified by their three-letter and one-letter codes. When using this to find out the amino acids coded for by DNA codons on the strand that is not transcribed (coding strand), change U to T

Remember

See Unit 1 to remind yourself about the cell structures that are involved in protein synthesis: ribosomes, RER and the Golgi body.

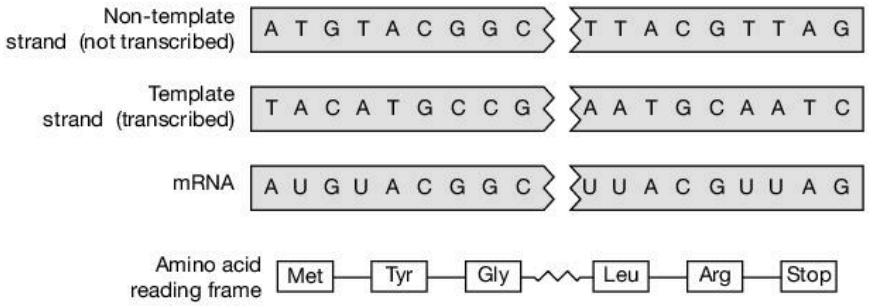
Key terms

Protein synthesis: production of proteins in cells involving **transcription** of DNA to produce mRNA, **translation** of mRNA in ribosomes to produce **polypeptides** and post-translational changes to polypeptides to form **proteins**.

Transcription: production of mRNA by assembly of **nucleotides** on a **template** polynucleotide of DNA.

Translation: the assembly of amino acids on ribosomes using sequences of **codons** in mRNA to determine the sequence of amino acids in a polypeptide.

Why 64 codons? You can count the number in the RNA wheel in Figure 6.5 or calculate it as 4^3 ($4 \times 4 \times 4$).



▲ **Figure 6.6** Follow the sequence of bases in the two polynucleotide strands of DNA and in the single mRNA polynucleotide for heptapeptide 1 – a compound found in the venom of the male duck-billed platypus

To use the RNA wheel, find the first base, e.g. U, then the second, e.g. A, and then the third, e.g. C. This mRNA codon (UAC) codes for the amino acid tyrosine. The DNA codon in the non-template strand is the same as in mRNA – TAC. Now find the complementary base sequence in the template strand of DNA – this will be ATG (remember T is used in DNA, not U).

Features of the genetic code

- Universal – it is found in all organisms (with a few minor variations).
- It codes for the 20 amino acids found in proteins.
- A sequence of three nucleotide bases codes for an amino acid.
- It is a non-overlapping code: each triplet is 'read' separately, as in Figure 6.6.
- There are three STOP codons: as a STOP codon does not specify an amino acid, it will act as a 'chain terminator'.
- There are between one and six codons for each amino acid (Met has one and Arg has six).
- There are more codons than needed so the genetic code is described as a degenerate code.
- The sequence of bases in the strand of DNA that is not transcribed is the same as the mRNA (expect that T in DNA codons is replaced by U in RNA codons).

Transcription

Each cell has only two copies of each gene, one on each homologous chromosome. In some cases, only one of those copies is able to code for a functioning version of the polypeptide. The cell may need to produce large quantities of the polypeptide. The DNA cannot pass out through the nuclear pores and provide information to all the ribosomes, so transcripts (copies) of the gene are produced in the form of mRNA by the process of transcription.

Transcription occurs in the nucleus of eukaryotic cells. For transcription to occur only the section of the DNA corresponding to the gene must uncoil and the hydrogen bonds must break so that the template strand is exposed.

The enzyme RNA polymerase moves along the template strand in the 3′–5′direction to synthesise a molecule of mRNA (Figure 6.11). The enzyme ensures that complementary base pairing occurs between the free nucleotides and the bases on the template strand so that mRNA has the same base sequence as the non-template (coding) strand. RNA polymerase catalyses the formation of phosphodiester bonds to form the sugar-phosphate backbone of mRNA.

Exam tip

You do not need to remember any of these codons, but you should be able to use the genetic code to find the amino acid that is coded by each codon and also the codons for any given amino acid.

Remember

The genetic code is all the codons you can see in the RNA wheel. It is *not* the sequence of bases in DNA or RNA that determines the sequence of amino acids in a protein.

Exam tip

A two-base code only specifies $4^2 = 16$ amino acids; a three-base code has $4^3 = 64$ triplets, which is many more than needed hence the description *degenerate*.

Link

Transcription factors bind to the region of DNA in front of the gene known as the promoter sequence (see Unit 16 page 190). Transcription factors 'switch on' genes during cell differentiation (see Unit 19 page 225).

The end product of transcription is an mRNA transcript. As there are many ribosomes, the cell is able to synthesise many copies of the polypeptide at the same time, so mRNA polymerase catalyses the synthesis of many mRNA molecules from the gene. These mRNA transcripts travel from the nucleus, through nuclear pores and into the cytoplasm.

mRNA molecules are short-lived. A cell's requirements change from minute to minute, so the cytoplasm contains ribonuclease enzymes that catalyse the hydrolysis of phosphodiester bonds in mRNA molecules. This releases nucleotides that can be reused for synthesis of RNA.

Transcription is similar to replication but only occurs on one strand of DNA. Along the length of a chromosome, some genes are transcribed from one polynucleotide, while other genes are transcribed from the opposite polynucleotide.

Exam tip

You should know the similarities and differences between replication and transcription, which are summarised in Table 6.3.

▼ **Table 6.3** A comparison between replication and transcription

Features	Replication	Transcription
DNA unwinds and hydrogen bonds within DNA break	✔	✔
number of polynucleotide strands that act as templates	2	1
free activated nucleotides align against DNA	✔ (DNA nucleotides - pentose is deoxyribose)	✔ (RNA nucleotides - pentose is ribose)
bases of the free nucleotides	adenine, guanine, cytosine, thymine	adenine, guanine, cytosine, uracil
base pairing	A–T, T-A, G-C and C–G (permanent)	A–U, T-A, G-C and C–G (temporary)
name of enzyme that catalyses the synthesis	DNA polymerase	RNA polymerase
type of polynucleotide produced	DNA	mRNA
quantity of DNA involved in a nucleus	all the DNA in the nucleus	only the DNA of the genes that are active at the time

Once in the cytoplasm, mRNA can be translated into the primary sequence of a polypeptide. Amino acids have first to be 'identified' or 'labelled' using the same three-base code. As amino acid molecules do not have bases, they are attached to molecules of tRNA in the process **amino acid activation**.

Remember

This is a good place to look back to Unit 2 page 17 to remind yourself of the structure of an amino acid.

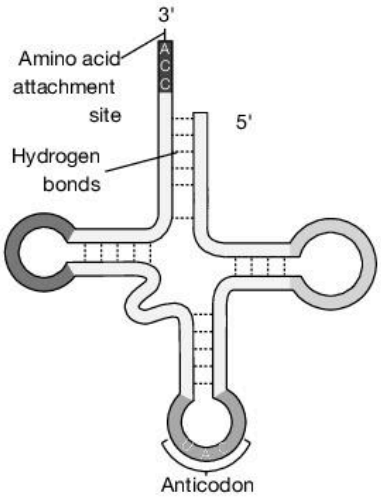
Amino acid activation

Enzymes in the cytoplasm have active sites that accept specific amino acids and specific tRNA molecules. The enzymes recognise the specific tRNA molecule for each type of amino acid. Energy is required for the attachment of an amino acid to its tRNA molecule. This is the only stage in protein synthesis in which the identity of the amino acid is important as after this it is identified by its tRNA molecule.

tRNA molecules have a shape resembling a clover leaf with the following regions:

- a site where amino acids are attached – always with the base sequence -CCA
- two 'loops' of nucleotides formed by some base pairing
- a 'loop' with an **anticodon** – the three bases that identifies the amino acid.

Figure 6.7 shows the specific tRNA molecule for methionine (Met) with the anticodon UAC. Methionine is attached to this tRNA molecule in an enzyme-catalysed reaction. The active site is specific to methionine and this tRNA molecule.



▲ **Figure 6.7** This is the tRNA molecule that carries the amino acid methionine to ribosomes. The anticodon is UAC, which pairs with the codon AUG on mRNA to start translation of all polypeptides

Translation

Now that there is a large supply of activated amino acids, the process of translation can begin. mRNA combines with the small sub-unit of a ribosome and then the large sub-unit joins so there is a 'groove' between the two. Two parts of the 'groove' allow amino acids to be brought close enough for a peptide bond to form between them:

- the A site accepts the tRNA–amino acid complex
- the P site holds the lengthening polypeptide.

As you read the text, follow the stages of translation in Figure 6.8.

Exam tip

A and P sites. A stands for amino-acyl and P for peptidyl. It is acceptable to know them as A and P sites.

To start the process, a tRNA–methionine complex enters the P site. The first codon (AUG) is the start codon and only a tRNA molecule with the anticodon UAC will form comlementary base pairs with it and therefore occupy the site. The A site is now exposed and another tRNA-amino acid complex enters the site. In Figure 6.8 the tRNA carries serine. If, by chance, another tRNA–amino acid complex enters the A site, then it does not pair and does not remain in place. Now that both sites are full, the enzyme peptidyl transferase in the ribosome catalyses the formation of a peptide bond between the C-terminal of methionine and the N-terminal of serine to join the two amino acids together.

Now the ribosome moves to the third codon. The first tRNA molecule leaves the ribosome and the second one carrying the dipeptide (Met–Ser) occupies the P site. A tRNA with the anticodon UGG occupies the now empty A site and another peptide bond forms between the dipeptide and glycine.

This process continues until the ribosome reaches a stop codon. There is no tRNA with an anticodon for this, so translation stops.

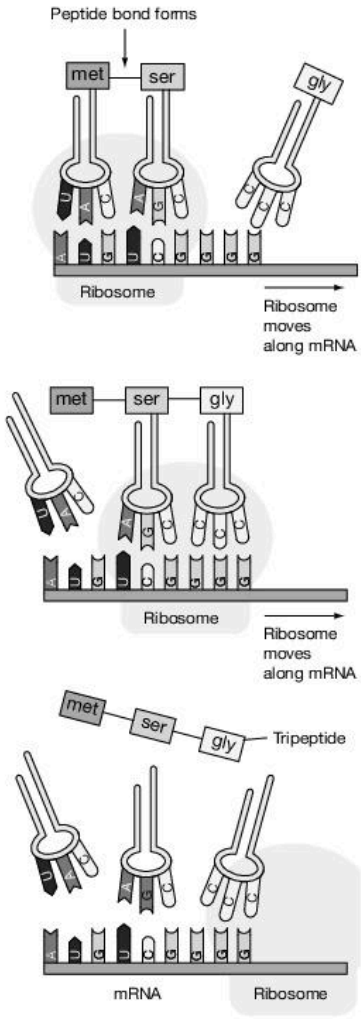
After translation on ribosomes on the rough endoplasmic reticulum (RER), polypeptides move through the lumen of the RER to the Golgi body. Polypeptides are modified while inside the RER and the Golgi body. They are folded into complex tertiary structures and may be combined with other polypeptides to form proteins with quaternary structure. Polypeptides often have sugar molecules attached to form glycoproteins.

Remember

The C-terminal of an amino acid is the end with the carboxyl group; the N-terminal is the end with the amino group (see Figure 2.12 on page 18). Peptides and polypeptides have C and N terminals as well.

Link

Follow the process carefully in the diagrams and then watch an animation of translation, such as those at www.johnkyrk.com, www.dnaftb.org or on YouTube.



▲ **Figure 6.8** Translation

Worked example

(a) State three ways in which transcription differs from replication.

The table shows the modes of action of three drugs that inhibit replication and protein synthesis.

Drug	Mode of action
A aphidicolin	inhibits DNA polymerase
R rifampicin (rifampin)	inhibits RNA polymerase
T tetracycline	prevents the attachment of t-RNA to the A site of ribosomes

(b) Explain the effect that (i) drug **A** has on replication, and
(ii) drugs **R** and **T** have on protein synthesis.

Answers

(a) In transcription, mRNA is made whereas in replication DNA is made. Only a small part of the DNA is used in transcription, whereas all the DNA is replicated. Activated nucleotides with ribose are used whereas in replication activated nucleotides have deoxyribose. In replication nucleotides with uracil are used. In replication nucleotides have thymine instead. Only one polynucleotide in DNA is the template whereas in replication both polynucleotides act as templates.

★ Exam tip

If you are asked for differences as in part (a) it is a good idea to give comparative answers as here.

(b) (i) **A** prevents DNA polymerase making a new DNA polynucleotide as complementary base pairing of free nucleotides with template nucleotides will not occur.

(ii) **R** prevents the formation of mRNA that is needed by ribosomes for translation.

T prevents the binding of each tRNA molecule carrying an amino acid to a ribosome so that peptide bonds cannot form.

Mutation

Mutation refers to changes in DNA. A change occurs in a single cell and is passed to all the descendants of that cell by DNA replication and mitosis.

Changes may occur that affect whole chromosomes or single genes.

- **Chromosome mutations** occur when a nucleus divides by mitosis or meiosis; these can change either the number of chromosomes in a nucleus or the structure of individual chromosomes.
- **Gene mutations** occur during DNA replication during interphase of the cell cycle; these are changes to the number or sequence of base pairs.

Key terms

Mutation: a change to a chromosome (chromosome mutation) or to a gene (gene mutation).

Gene mutation: a change to the nucleotide sequence of a gene.

Substitution mutation: a change to a nucleotide sequence in a gene in which one base pair is changed to another, e.g. A–T to T–A. or C–G to T–A.

Haemoglobin

The gene *HBB* codes for β-globin of haemoglobin. A **substitution mutation** can occur in the sixth codon of this gene. This has very severe consequences if all the β-globin polypeptides in haemoglobin are changed. The mutation involves a change of the base A to the base T in the sixth codon of the coding strand in *HBB*. This leads to the change from CTC to CAC in the template strand and hence the substitution of valine for glutamic acid in the sixth position of the primary structure of β-globin. This type of haemoglobin is known as sickle cell haemoglobin S (HbS) to distinguish it from the normal form, haemoglobin A (HbA).

The two variants, or **alleles**, of the gene *HBB* are known as Hb^A and Hb^S. Children who inherit two copies of the Hb^S allele develop sickle cell anaemia. There is more about the inheritance of these alleles in Unit 16.

Key term

Allele: a variant of a gene that is the result of a mutation. Alleles of the same gene are found at the same place on a chromosome.

Raise your grade

These two questions are similar to real Paper 2 questions as they deal with topics from several Units rather than just one. This is why it is important in your revision to make links between different topics.

1 (a) DNA and polypeptides are both polymers.

State two ways in which a molecule of DNA differs from a polypeptide. [2]

DNA is a double helix. ✔ but a polypeptide has only one 'strand' not two ✔ and it may have a helix shape but it cannot be a double helix.

Good attempt at explaining the difference between the whole molecules. It might be easier to state the differences in the monomers (nucleotides v amino acids) and the chemical composition (DNA has phosphate but the polypeptide does not).

A length of DNA with a specific nucleotide sequence codes for a polypeptide that is part of the receptor protein for the hormone insulin. The receptor protein is a glycoprotein found in the cell surface membrane of cells in the liver, muscles and fat storage tissue.

(b) State the name given to a length of DNA that codes for a polypeptide. [1]

Gene. ✔

(c) Outline how a sequence of nucleotides in DNA leads to the production of the polypeptide that is part of the receptor for insulin. [6]

The sequence of nucleotides is transcribed by RNA polymerase to form mRNA. ✔ This travels through the nuclear pores into the cytoplasm. mRNA combines with a ribosome and translation occurs. ✔ tRNA molecules bring amino acids to the ribosome and anticodons pair with complementary codons on the mRNA. ✔ e.g. the anticodon AAA pairs with the codon UUU. ✔ The amino acids are reacted together by a condensation reaction to form peptide bonds. ✔ A polypeptide forms and moves via the ER to the Golgi body ✔ where it is modified by glycosylation so it has sugars attached to some of the amino acids.

Good answer that gives a brief overview of the whole process. The inclusion of an example of anticodon–codon binding was a good idea.

(d) Receptors for hormones such as testosterone are in the nucleus rather than on the cell surface membrane.

Suggest why the receptor for insulin is on the cell surface, but the receptor for testosterone is in the nucleus. [3]

Insulin is a globular protein and therefore water soluble as it is transported in the blood plasma, which is mostly water. ✔ Insulin cannot pass through the phospholipid bilayer and there is no channel or carrier protein for it. ✔ Therefore, cells have cell surface receptors for insulin. Testosterone is a steroid (a type of lipid) so can pass through the phospholipid bilayer by simple diffusion. ✔

The candidate has given a very thorough answer.

2 In the 1950s, Erwin Chargaff in Paris determined the relative quantities of the four bases in DNA in different organisms. The results for five species, summarised in the table below, provided evidence for the structure of DNA proposed by James Watson and Francis Crick in 1953.

Organism	Percentage of adenine	Percentage of thymine	Percentage of cytosine	Percentage of guanine
Escherichia coli (bacterium)	24.7	23.6	25.7	26.0
a yeast	31.3	32.9	17.1	18.7
wheat	27.3	27.1	22.8	22.7
chicken	28.0	28.4	21.6	22.0
human	29.3	30.0	20.0	20.7

(a) (i) Calculate the ratios of A:T and of C:G for *Escherichia coli* and for a human. [2]

organism	ratio of A:T ✔	ratio of C:G ✔
E. coli	1.05:1	0.99:1
human	0.98:1	0.97:1

A good idea! Tabulating your answers to questions like this is perfectly acceptable even if there is no table provided on the exam paper.

(ii) Explain how the ratios you have calculated provided evidence for the structure of DNA. [3]

The ratios are all very near 1:1 ✔ (as they are for the other species in the table as well). This supports the idea of base pairing in DNA ✔: A–T and C–G are the base pairs. ✔

The answer is clearly presented and the results in the table do provide evidence for base pairing in DNA.

(b) The ratios of A:T and C:G in mRNA do not show a pattern similar to those in the table above. Explain why this is so. [3]

DNA is a double helix made of two strands that are attached to each other by hydrogen bonds. The bonds are between the base pairs A+T and C+G. ✔ There is no base pairing in mRNA ✔ as it is composed of a single polynucleotide made by transcribing one of the strands of DNA. ✔

A full explanation. The candidate could say that it is the template strand that is transcribed.

(c) (i) Calculate the ratio of A+T:C+G for yeast and for wheat. [2]

yeast 1.79:1 ✔ wheat 1.20:1. ✔

Correct answers.

(ii) Explain why the ratio of A+T:C+G differs from species to species. [2]

These ratios are different because there are different numbers of base pairs A–T and C–G. The numbers of A and T do not depend on the numbers of C and G as they do within their base pairs (A–T and C–G). ✔ Different species have different genes with different sequences of bases to code for the different proteins that they make. ✔

A very comprehensive explanation.

Exam-style questions

1 During transcription nucleotides are assembled along the template strand of DNA to form mRNA.

In a DNA molecule, the triplet CAG on the template strand codes for the amino acid valine.

What is the base sequence of the anticodon on the tRNA to which valine becomes attached?

A CAG

B CUG

C GTC

D GUC [1]

2 The DNA codons for glutamic acid are GAA and GAG. Two DNA codons for valine are GTG and GTA. In sickle cell anaemia, valine is present in the sixth position in β-globin instead of glutamic acid.

Assuming that a single base pair substitution has occurred, what is the mRNA codon for the amino acid in the sixth position of β-globin in people with sickle cell anaemia?

A CUC

B CUT

C GUG

D GUU [1]

3 A DNA molecule is replicated to form two molecules of DNA. Which statement about the polynucleotides of the newly replicated DNA molecule is correct?

A both polynucleotides are made of newly polymerised nucleotides

B both polynucleotides contain bases from the original molecule

C one polynucleotide is new and the other was part of the original molecule

D the base pairs are conserved and have new sugar-phosphate backbones [1]

4 The proportion of guanine in a sample of DNA is 22%. What proportion of the bases are adenine?

A 22%

B 28%

C 44%

D 56% [1]

5 Which describes a codon?

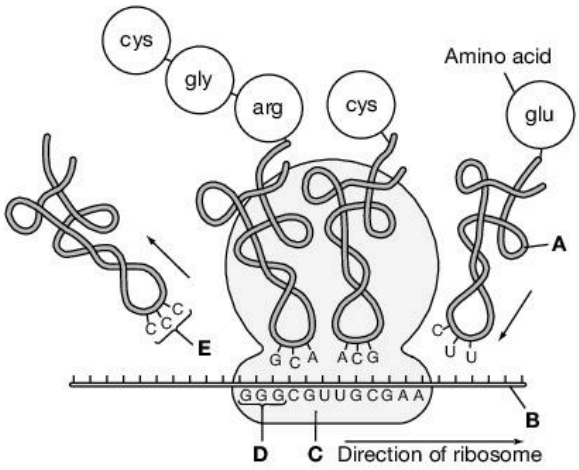
A a part of DNA or mRNA which codes for a specific amino acid

B a part of DNA which codes for a particular polypeptide

C a part of mRNA that codes for three amino acids

D a part of a tRNA molecule that binds to mRNA [1]

6 The diagram shows the part of the process of protein synthesis that occurs in the cytoplasm of eukaryotic cells.



(a) Name the types of RNA labelled **A**, **B** and **C**. [3]

(b) State the terms used to describe the groups of nucleotide bases at **D** and **E**. [2]

(c) Explain how amino acids become arranged into the correct sequence in the primary structure of the protein. (You may refer to the diagram above to help you with your answer.) [5]

(d) Describe three features of a polypeptide molecule that are **not** found in a DNA molecule. [3]

extra questions available online

7 Transport in plants

Key points

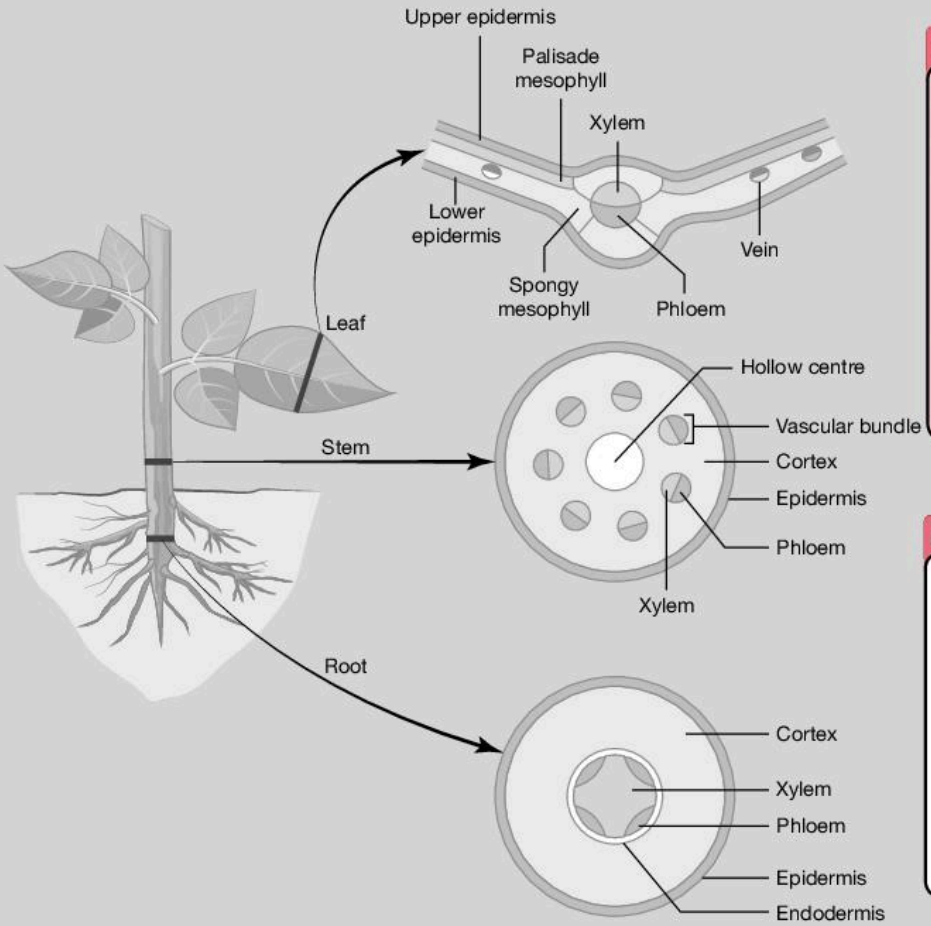
- ☐ Draw and label from prepared slides: plan diagrams to show the distribution of tissues in transverse sections of stems, roots and leaves of herbaceous dicotyledonous plants; and cells in the different tissues in transverse and longitudinal sections of stems, roots and leaves.
- ☐ Recognise xylem vessel elements, phloem sieve tube elements and companion cells in prepared slides and in photomicrographs.
- ☐ Describe the structure of xylem vessel elements, phloem sieve tubes elements and companion cells and relate their structures to their functions.
- ☐ Explain the movement of water between plant cells, and between them and their environment, in terms of water potential.
- ☐ Explain how the hydrogen bonding of water molecules is involved with movement in the xylem by cohesion-tension in transpiration pull and by adhesion to cellulose cell walls.
- ☐ Describe the pathways and explain the mechanisms through which water and mineral ions are transported from soil to xylem and from roots to leaves, including references to the symplastic pathway, the apoplastic pathway and the Casparian strip.
- ☐ Explain the mechanisms that are responsible for the absorption of water and ions and their transport from roots to leaves.
- ☐ Define and explain transpiration in plants.
- ☐ Investigate experimentally the factors that affect transpiration rate (using simple potometers, leaf impressions, epidermal peels, and grids for determining surface area) and explain their effects.
- ☐ Make annotated drawings, using prepared slides of cross-sections, to show how leaves of xerophytic plants are adapted to reduce water loss by transpiration.
- ☐ State that assimilates (e.g. sucrose and amino acids) move between sources (e.g. leaves, storage organs) and sinks (e.g. buds, flowers, fruits, roots, storage organs) in phloem sieve tubes.
- ☐ Explain how sucrose is loaded into phloem sieve tubes by companion cells using proton pumping and the co-transporter mechanism in their cell surface membranes.
- ☐ Explain mass flow in phloem sap down a hydrostatic pressure gradient from source to sink.

Practical skills

Plan diagrams of plant organs

Flowering plants have three organs: stem, root and leaf. All other organs are modifications of these three. Dicotyledonous plants have two cotyledons or 'seed leaves'. Herbaceous plants do not have wood in their roots and stems.

You must be able to recognise the cross-sections of roots, stems and leaves of herbaceous dicotyledons. You are expected to draw plan diagrams to show the distribution of tissues in these sections. Microscope slides and/or photomicrographs in Paper 3 could be other species that you have not seen before.



▲ **Figure 7.1** Simple plan diagrams showing the distribution of tissues in the root, stem and leaf of an herbaceous dicotyledonous plant

Exam tip

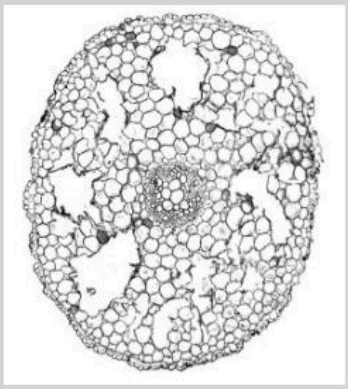
Expect to see plan diagrams in Paper 1 or Paper 2 and expect to name the different tissues shown in Figure 7.1. Do *not* draw plan diagrams like these in Paper 3 – draw what you see in the slides or photomicrographs as in Figures 7.3, 7.7 and 7.10.

Link

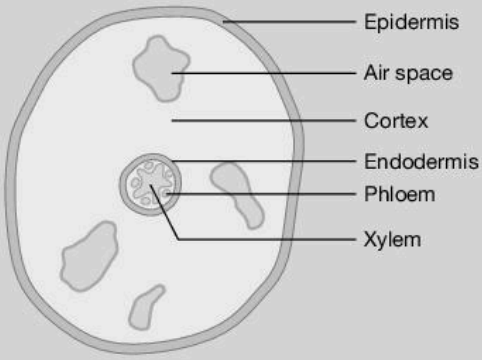
Plan diagrams show the distribution and relative proportions of the different tissues in organs. They never show any cells. In Unit 20 Practical assessment page 242 you will find some advice about how to draw plan diagrams.

Root

The central vascular tissue (**xylem** and **phloem**) in roots is in the centre of the root; in young roots, the xylem in the centre has a cross-like or star-like appearance.



▲ **Figure 7.2** A Photomicrograph of a cross-section of a root of buttercup, *R. repens* ×25

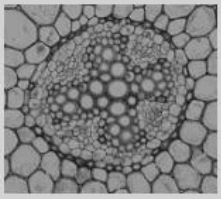


▲ **Figure 7.3** A plan diagram of the cross-sections in Figures 7.2 and 7.4 ×25

Key terms

Xylem tissue: plant tissue that transports water and ions from roots to the aerial parts of a plant and provides support.

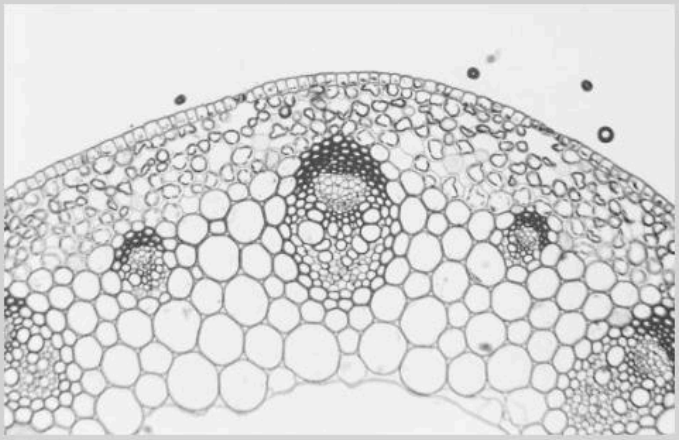
Phloem tissue: plant tissue that transports organic compounds which have been made in leaves or storage organs to other parts of the plant.



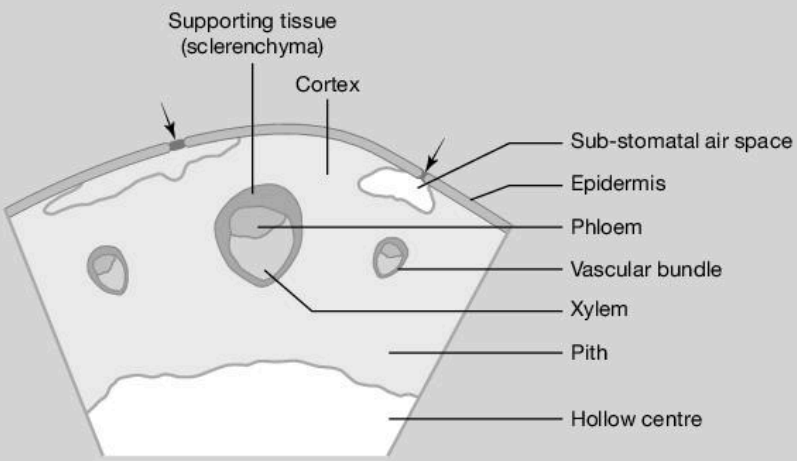
▲ **Figure 7.4** A cross-section of the central region of a root of buttercup, *R. repens* ×70

Stem

The vascular tissue in stems is arranged into vascular bundles that are between the central pith and the outer cortex (Figures 7.5 and 7.6). Xylem is always nearer to the central part of the stem relative to the phloem. (Figure 7.7).



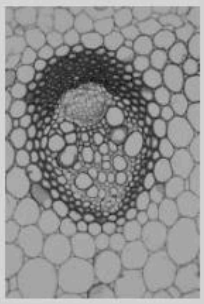
▲ **Figure 7.5** Part of a cross-section of a stem of buttercup, *R. repens*. Five vascular bundles are visible ×50



▲ **Figure 7.7** A plan diagram of the cross-section in Figure 7.5. The arrows indicate the positions of stomata ×25

Exam tip

When looking at any biological structure you should think about the functions that it carries out and how its structure is related to those functions.



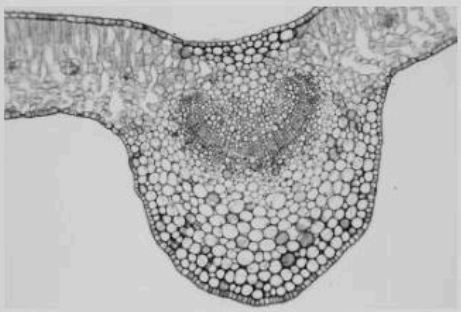
▲ **Figure 7.6** A cross-section of a vascular bundle of buttercup, *R. repens* x50

Link

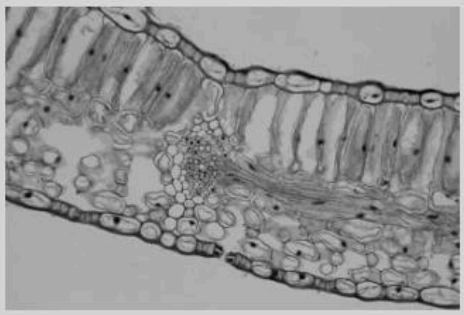
Cross-sections of roots may have root hairs, which are outgrowths of epidermal cells that increase the surface area for absorption. See Unit 4 for more about the movement of substances into and out of cells.

Leaf

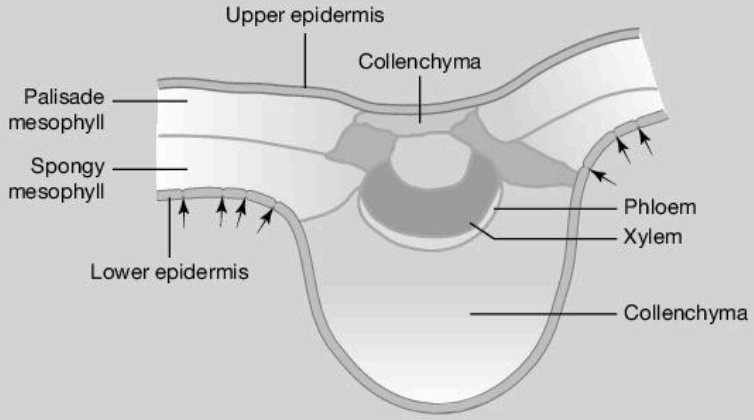
The vascular tissue in leaves is arranged into vascular bundles that are within the spongy mesophyll. The xylem is always nearer to the upper leaf surface than the phloem.



▲ **Figure 7.8** A cross-section of the central part of a leaf of privet, *Ligustrum* sp. This shows the main vein that forms the midrib – the central part of the leaf ×10



▲ **Figure 7.9** A cross-section of a leaf of *Helleborus*. The section has cut along a vein at a point where it branches. Some xylem vessels are visible to the right of the cross-section of the vein. There is an open stoma on the lower surface with the two guard cells on either side ×40



▲ **Figure 7.10** A plan diagram of the cross-section in Figure 7.8. The arrows indicate the positions of stomata ×12

Exam tip

A vein in a leaf is composed of xylem and phloem and supporting tissue. Do not confuse them with veins in animals which carry blood towards the heart.

Transport of water and ions in plants

There are four aspects to transport in plants:

- absorption of water and ions from the soil
- movement of water and ions over short distances within organs (roots, stems and leaves), e.g. from root hairs to xylem and from xylem to mesophyll cells in leaves
- long distance transport of xylem sap from roots to all other parts of a plant and phloem sap from leaves and storage organs to the rest of the plant
- loss of water vapour from leaves to the atmosphere.

Roots have epidermal cells with root hairs that increase the surface area for absorption.

Root hairs are near the root tips where the epidermis is permeable. They are very thin so they can extend between soil particles; they have thin cellulose cell walls and there is no cuticle, so diffusion distances are short. The cell surface membranes of root hair cells have carrier proteins and channel proteins for absorption of ions; they also have many **aquaporins** (page 42).

The concentration of ions in the soil is very low, so plants use active transport to absorb them.

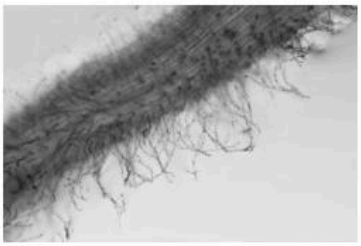
Water moves from the soil down a water potential gradient by osmosis into the root hair cells mainly through the aquaporins in the cell surface membranes.

The pathway taken by water and ions from root hair cells to the xylem in the centre of the root is the one of least resistance. There are plenty of cell walls and intercellular spaces through which water and ions can pass without having to go across cell surface membranes – apoplastic pathway. Some water and ions will enter cells and pass from cell to cell through the interconnecting **plasmodesmata** – symplastic pathway. Some water may also travel through the tonoplast into the vacuole of each cell on its way across the cortex of the root. This **vacuolar pathway** is part of the symplastic pathway.

Key terms

Apoplastic pathway: the pathway taken by substances moving through the cell walls and intercellular spaces in a plant tissue.

Symplastic pathway: the pathway taken by substances moving through plant tissues in which substances travel from cell to cell through **plasmodesmata** so avoiding crossing cell surface membranes.



▲ **Figure 7.11** Root hairs of thyme, *Thymus* sp. ×30

Link

Aquaporins, the special channel proteins for water, are described in Unit 4 (see page 42).

Remember

Facilitated diffusion and active transport both use carrier proteins. Remind yourself about these methods of movement across cell membranes in Unit 4.

Remember

At this point you should revise osmosis and water potential gradients from Unit 4.

Remember

Plasmodesmata are described in Unit 1. Do *not* confuse them with channel proteins in cell membranes, such as aquaporins.

The central vascular tissue in roots is surrounded by the **endodermis**, a single layer of cells of the cortex that controls the movement of ions from the cortex to the xylem. The cell walls contain **suberin** which is an impermeable, waxy substance that does not allow water and ions to flow *between* the cells. Instead, everything has to travel *through* the cytoplasm of the cells. In young roots, this suberinised band is visible in cross-sections of the root and is known as the **Casparian strip**. This allows cells of the endodermis to control what passes into the central vascular tissue.

Xylem vessel elements

Xylem vessels are made of specialised cells, **xylem vessel elements**, which differentiate from meristematic cells. These cells thicken the side walls with cellulose and with lignin, a complex compound that waterproofs the walls and gives them strength.

The end walls are not thickened. When water starts to flow through the column of these cells, the end walls break apart to form a continuous column without any cytoplasm or end walls to create resistance. As xylem vessels are dead and have no cell contents and no cell surface membranes, they are part of the apoplastic pathway.

Key terms

Endodermis: the inner layer of cells of the cortex that surrounds the vascular tissue in roots and stems.

Casparian strip: a band of suberin in endodermal cell walls that prevents the flow of water and ions between cells, so blocking the apoplastic route.

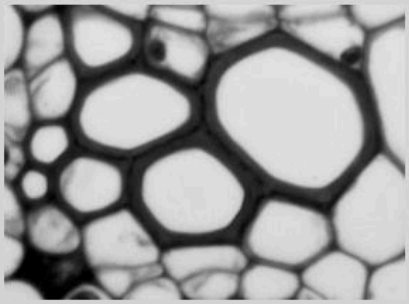
Xylem vessel: a column of **xylem vessel elements** for transport of water and ions in plants.

Xylem vessel element: a cell that forms part of a xylem vessel.

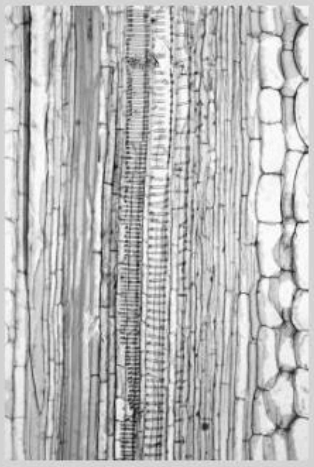
Practical skills

The structure of xylem vessels

You can recognise xylem vessels as they have thick cell walls, wide cells and no cell contents (Figures 7.12 and 7.13). In sections prepared for the microscope they are often stained to locate the cells with lignin. Their cell walls are often stained red or bright blue (see Unit 20 Practical assessment page 241–242).



▲ **Figure 7.12** Xylem vessels from the central vascular tissue of a root in cross-section ×400



▲ **Figure 7.13** Xylem vessels with spiral thickening in the centre of a longitudinal section of a stem ×25

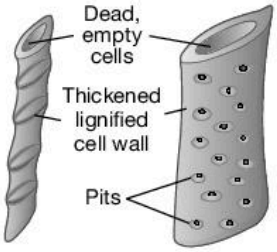
Adaptations of xylem vessels

Feature that makes cell walls impermeable to water so it remains in the xylem vessels:

- walls impregnated with lignin.

Features that allow flow of water throughout the plant:

- xylem vessel elements are arranged in columns, forming 'tubes' that extend from roots to leaves
- cellulose walls are hydrophilic so water molecules can form hydrogen bonds to them so that columns of water are supported.



▲ **Figure 7.14** The adaptive features of xylem vessels

Features that give a low resistance to the flow of water in the xylem vessels:

- wide lumen, up to 0.7 mm
- no cell contents – no membranes, cytoplasm or nucleus
- no end walls separating the xylem vessel elements
- xylem vessels are continuous columns.

Movement in the stems to the leaves and the air

The gas exchange surfaces within leaves are the surfaces of the palisade and spongy mesophyll cells that are in contact with air. They are damp because water diffuses into them from the interior of cells and because water moves directly through cell walls from the xylem. Water evaporates from the water films in the cell walls. This makes the air spaces throughout the leaves fully saturated with water vapour. The relative humidity inside the air spaces is always 100%. If the atmosphere has a lower relative humidity, then water vapour will diffuse out through the stomata. This loss of water vapour from plants is **transpiration**.

Transpiration leads to the loss of large quantities of water vapour because of the extensive gas exchange surface inside leaves. When stomata are open, water vapour can easily diffuse into the atmosphere.

Transpiration pull

Loss of water vapour from the aerial surfaces of a plant causes water movement through the plant – the energy to evaporate water comes from the Sun. Transport in the xylem by **transpiration pull** is a passive process for the plant.

The loss of water vapour from the cell surfaces causes the water molecules to move away from the cell surfaces deeper into the cell wall. Cellulose is hydrophilic and attracts water by hydrogen bonding. This attraction of water to a surface where water is in contact with the air exerts a pulling action on water into the cell and through the apoplast all the way to the xylem in the leaf. This is because of the forces of cohesion between water molecules – the result of hydrogen bonding. The 'pull' from the leaves driven by the evaporation of water and the cohesive forces between water molecules is the **cohesion-tension** mechanism that results in transpiration pull.

Remember

This is a good place to remind yourself about hydrogen bonding from Unit 2.

Water molecules 'stick' together by cohesion and they also 'stick' to the cellulose lining the walls of xylem vessels by **adhesion**. Both are important in maintaining a flow of water in the narrow xylem vessels in pulling water across the small spaces in the cell walls between xylem and other tissues.

You can explain the mechanism of water transport in xylem in steps.

Key terms

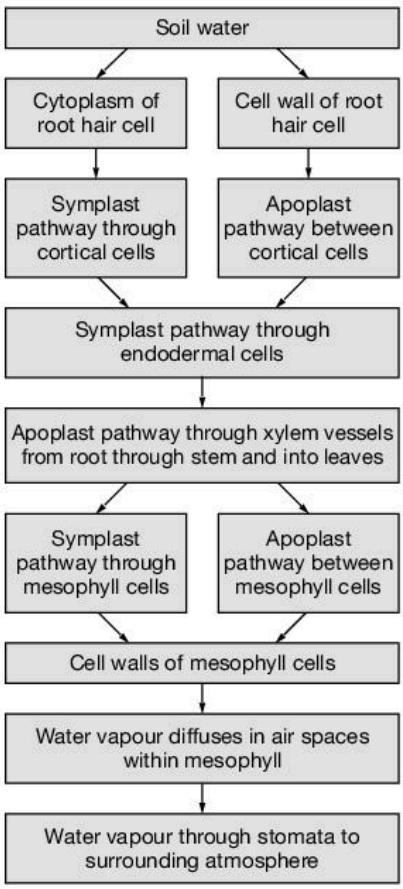
Transpiration pull: loss of water by transpiration causes flow of water through the xylem by **cohesion-tension** from roots to leaves.

Cohesion-tension: the mechanism by which water moves through the xylem. The tension is set up by transpiration from leaves and this is transmitted to columns of water by the force of attraction (cohesion) between water molecules.

Key terms

Transpiration: the loss of water vapour by diffusion from the aerial parts of plants such as the leaves and most stems.

Transpiration stream: the continuous movement of sap in the xylem.



▲ **Figure 7.15** A flow chart showing the pathway taken by water as it moves from the soil, through the plant to the atmosphere

Exam tip

Drawing flow charts is a good way to learn the correct sequence of events in a biological process, e.g. water movement in plants.

- Loss of water *vapour* by diffusion, mainly through stomata although there is some loss through the cuticle (cuticular transpiration).
- Evaporation of water from the cell walls of mesophyll cells.
- Cohesion-tension acts to pull water across the apoplastic pathway from the xylem in the leaf. Water may also move down the water potential gradient from the cells to the cell surfaces. This decreases the water potential of the cells, helping to move water from the apoplast.
- Cohesion-tension acts to pull water upwards in the xylem vessels.

Rates of transpiration fluctuate

Rates of transpiration are dependent on the size and the density of stomata per unit area and the location of the stomata, which are controlled by guard cells. These cells are sensitive to light intensity, humidity, temperature and carbon dioxide concentrations inside leaves. They are also sensitive to signalling chemicals, released by the plant when water is in short supply. Stomata tend to be closed at night and open during the day, although there are some species where this rhythm is reversed.

Exam tip

The size of the opening of stomata is described as the width, the diameter or the aperture. The maximum aperture depends on the species, but may be as much as 20–30 µm in plants such as *Tradescantia* (see Question 2 page 87).

Link

There is more about stomata and the way they control the diffusion of gases into and out of leaves in Unit 14 page 167.

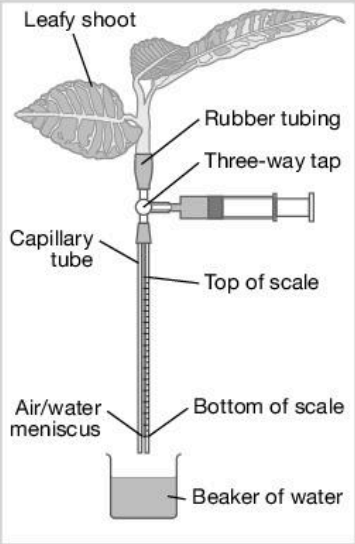
Practical skills

Using potometers to measure rates of water uptake

A simple way to measure water uptake by plants is to use the apparatus in Figure 7.16. The volume of water absorbed by the shoot can be measured by marking the levels of water on the flask and finding out how much has been lost. The balance is used to measure the mass of water lost from the leafy shoot to the atmosphere by transpiration. A layer of oil on the water in the flask prevents evaporation from the water surface.

▲ **Figure 7.16** A mass potometer

The potometer in Figure 7.17 is a quicker way to measure water uptake. Shoots are attached to capillary tubing so it is possible to measure the absorption of tiny volumes of water in a short time.



▲ **Figure 7.17** This simple potometer can be made simpler by omitting the three-way tap and syringe

Determining rates of transpiration

Record the distance travelled by the meniscus over a specific time and calculate the rate of water uptake. Use the formula $\pi r^2 h$, where r is the radius of the bore of the capillary tubing and h is the distance travelled in mm. Divide the volume in mm^3 by the time in minutes or seconds to give a rate in $mm^3 min^{-1}$ or $mm^3 s^{-1}$.

Rates of transpiration are often expressed as mass of water lost per unit leaf area per unit of time. You can measure the area of leaves by putting some leaves on a piece of graph paper, drawing around the leaves and then counting the squares that are more than half-filled by the leaves. Multiply by two if the total surface area of the leaf is required.

You can determine the stomatal density (number of stomata per unit area of leaf) by: pulling off a layer of epidermis, putting the epidermal peel into a drop of water on a slide and adding a cover slip; or taking an impression by painting a leaf with nail varnish or 'new skin' liquid plaster, leaving it to dry and then attaching it to a slide with transparent sticky tape.

Count the number of stomata in a field of view. Use a calibrated eyepiece graticule to measure the area of the field of view. Repeat for several fields of view to calculate the mean number of stomata per unit area.

Factors that affect transpiration

Potometers are used to determine the effect of different environmental factors on the rate of transpiration. When investigating each factor, you should keep the other factors constant or measure all the factors so that you can assess whether they affect your results.

Rates of transpiration are influenced by the degree of opening of stomata and various environmental factors. Light intensity is the main factor that determines whether stomata are open or not. It also determines the width of the stomata (stomatal aperture). The rate of diffusion of water vapour through stomata is determined by the water potential gradient between the inside of the leaf and the outside. The gradient becomes steeper if the humidity *decreases*, the temperature *increases* or the wind speed *increases*.

Exam tip

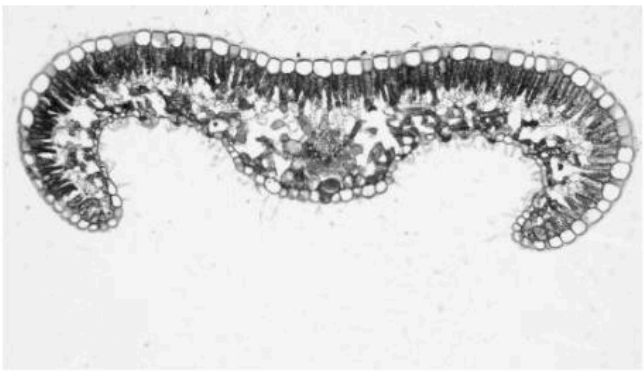
Temperature and wind speed were measured in the investigation described in Question 1 on page 85.

Xerophytes

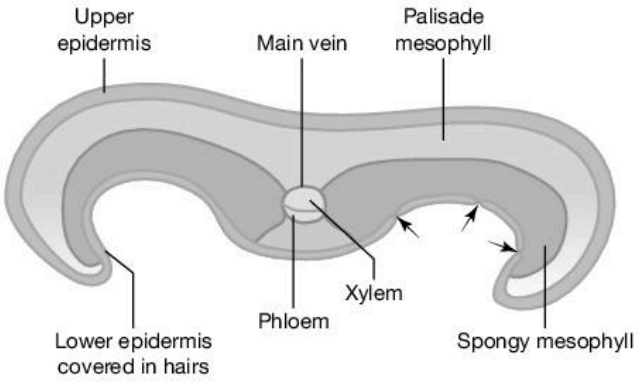
Xerophytes are plants that are adapted to living in places where there is a shortage of water. Their leaves have various adaptations to *reduce* water loss.

▼ **Table 7.3** Some adaptations of leaves of xerophytes

Feature	Adaptation for reduction of water loss
leaf permanently rolled or rolls in dry conditions	air is trapped inside the rolled leaf; water vapour diffuses into the air, but is lost slowly to the atmosphere. The humid air is trapped and reduces the diffusion of water vapour through the stomata, as the water potential gradient is less steep. Leaves that do this have all their stomata facing inwards when the leaf is rolled
epidermal cells with thick walls	increases distance for diffusion of water vapour
thick cuticle	cuticle is made of waxy substances that waterproof the leaf and reduce uncontrolled water loss
leaf covered in trichomes (hairs)	hairs trap a layer of still, humid air; this reduces diffusion of water vapour from the interior of the leaf as the water potential gradient is less steep
low stomatal density	fewer ways for water vapour to diffuse out of the leaf
stomata sunken in pits or grooves in the leaf	still, humid air collects in the pits and grooves; this reduces the diffusion of water vapour through the stomata to the outside



▲ **Figure 7.18** A cross-section of a leaf of cross-leaved heath, *Erica tetralix*. Water loss is reduced by: a thick cuticle on the upper epidermis, hairs on the lower epidermis, and slightly curved leaves so that all the stomata open into a groove on either side of the main vein



▲ **Figure 7.19** A plan diagram of the leaf in Figure 7.18. The arrows indicate the position of stomata

Movement in the phloem

The movement of phloem sap from **source** to **sink** is known as **translocation** (meaning from one place to another). Phloem transports **assimilates**, which are compounds produced by the metabolism of a plant. Sucrose and amino acids are produced in mesophyll cells and transported across the leaf into the nearest fine endings of the phloem sieve tubes.

Phloem transports assimilates produced by mature, photosynthesising leaves to regions where growth occurs in roots, stems, flowers, fruits and seeds and also to storage organs, such as root and stem tubers. Storage organs store energy as starch for survival over very dry or very cold periods. When growth begins again, these stores are mobilised and sucrose and amino acids are sent to new shoots and young leaves that are not yet photosynthesising. Some organs can be both sources and sinks in phloem transport at different times in the growth of a plant.

The conducting cells in phloem form **sieve tubes**, which are made from specialised cells known as **sieve tube elements**. These cells differentiate from meristematic cells that divide longitudinally to form two cells of different sizes. The larger cells are sieve tube elements and the smaller cells are companion cells. Sieve tube elements lose their nuclei and much of the cytoplasm, but companion cells retain their nuclei and have a dense cytoplasm with many mitochondria to provide energy.

Key terms

Source: any plant organ that makes substances and loads them into phloem, e.g. leaves and storage organs.

Sink: any plant organ that unloads substances from the phloem and uses them in its metabolism, e.g. storage organs, young leaves, stems.

Assimilates: substances produced by a plant's metabolism, e.g. sucrose and amino acids.

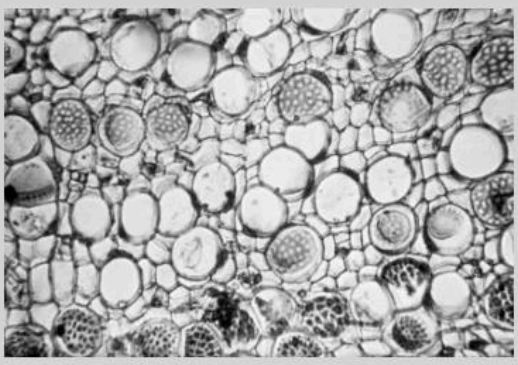
Key terms

Phloem sieve tube: a long column of **sieve tube elements** that transport organic compounds in plants.

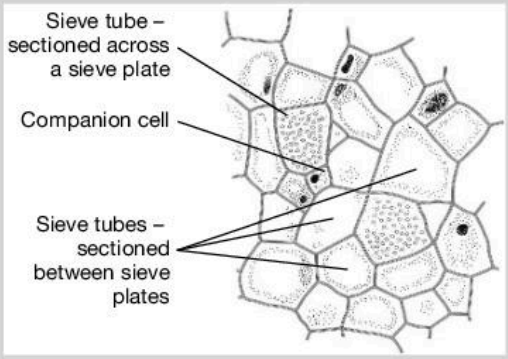
Phloem sieve tube element: a cell that forms part of a sieve tube.

Practical skills

Recognising phloem sieve tube elements and companion cells from light micrographs



▲ **Figure 7.20** A cross-section of phloem tissue from a vascular bundle in a stem of a squash plant, *Cucurbita pepo* showing phloem sieve tubes ×350



▲ **Figure 7.21** A drawing of the phloem tissue showing the position of sieve tube elements, sieve pores and companion cells ×400

You can recognise phloem sieve tubes as their cell walls are not as thick as xylem cell walls and they are usually adjacent to the much smaller companion cell. Some sections have sieve plates (see Figure 7.20 and Figure 7.21).

Sieve plates are perforated end walls that are thought to prevent sieve tubes expanding because of the high hydrostatic pressures that develop within them. There are many plasmodesmata between sieve tubes and companion cells, in sources and sinks where assimilates, such as sucrose, are loaded and unloaded.

▶ **Figure 7.22** A longitudinal section of phloem tissue from *Cucurbita pepo* showing sieve tubes with sieve plates ×30

Adaptations of phloem sieve tubes

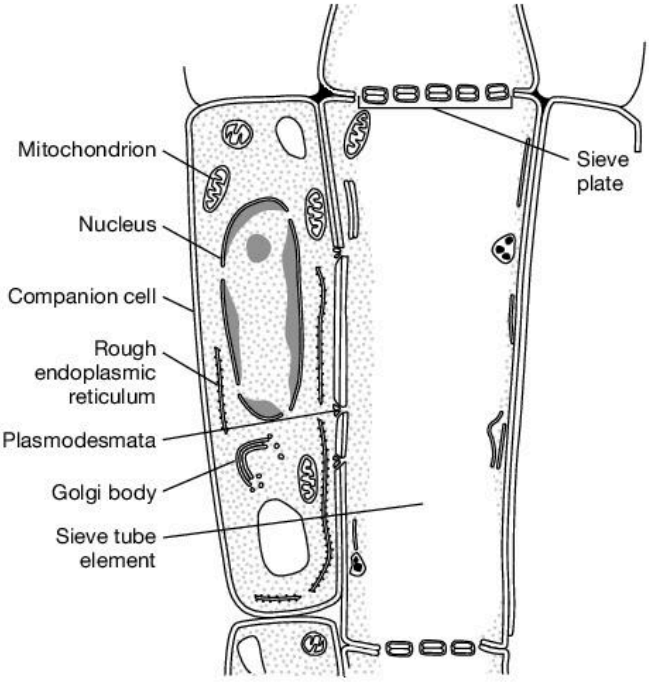
Phloem tissue is adapted for the transport of phloem sap.

Sieve tube elements have:

- cell surface membranes that retain sucrose and other assimilates within the cells
- few cell contents to reduce resistance to flow of phloem sap
- sieve plates to hold sieve tubes together and resist any internal pressure
- sieve pores to allow ease of flow between sieve tube elements.

Companion cells have:

- many mitochondria to provide energy to move solutions into the sieve tubes
- many plasmodesmata to allow easy movement of phloem sap into and out of the sieve tubes
- pump proteins and co-transporter proteins in the cell surface membranes for absorption of sucrose from the apoplast pathway from mesophyll cells
- some plasmodesmata shared with mesophyll cells for transport of sucrose via the symplast pathway (in some species).



▲ **Figure 7.23** This drawing was made from transmission electron micrographs. It shows a sieve tube and a companion cell in longitudinal section

Mechanism of translocation

Phloem sap may move in either direction in a plant. For example, on a hot, bright day that has good conditions for photosynthesis, phloem sap moves downwards from leaves to roots and also upwards from leaves to growing points, flowers, seeds and fruits. Xylem sap moves only in one direction as it is pulled upwards by transpiration. During the life of a plant, phloem sap may travel in both directions through an individual sieve tube. When a leaf starts to grow, it is not photosynthesising but using sucrose imported from other leaves to provide its cells with energy. Later, when the leaf is photosynthesising, it becomes a net exporter of sucrose, which will then flow through sieve tubes in the opposite direction to that at the start.

There are three main principles involved with transport in the phloem.

- Sucrose and other assimilates are loaded at the source where there is a build up of hydrostatic pressure.
- A pressure gradient is responsible for movement of phloem sap through sieve tubes from source to a sink.
- Sucrose and other assimilates are unloaded at the sink so forming a low hydrostatic pressure.

The mechanism for phloem transport is **mass flow**, which is achieved by hydrostatic pressure gradients between sources and sinks. This is also known as pressure flow.

★ Exam tip

Make sure you fully understand how osmosis and water potential gradients are involved in phloem transport. Osmosis is essential for the creation of the pressure gradient between source and sink.

Key term

Mass flow: the movement of a fluid in a transport system. The fluid moves in one direction within vessels (e.g. xylem vessels and phloem sieve tubes in flowering plants).

Worked example

(a) Describe the function of the following plant cells; in each case, relate the structure of the cell to its function.

(i) Endodermal cell.

(ii) Sieve tube element.

(iii) Companion cell.

(iv) Xylem vessel element.

(b) Explain the terms *source* and *sink* as applied to transport in plants.

(c) Explain how the transport in the phloem differs from transport in the xylem.

Answers

(a) (i) Endodermal cells provide an impermeable barrier to water movement through the apoplastic pathway. There is a Casparian strip in the cell wall made of suberin (a waxy substance) that ensures water moves through cell membranes into the cells.

(ii) Sieve tube elements are elongate cells, joined end to end to form sieve tubes that transport phloem sap containing assimilates such as sucrose. The end walls are perforated so sap flows unhindered. The cells have very little cytoplasm so there is little resistance to the flow of sap.

(iii) Companion cells load sucrose into the sieve tube elements. Proteins in the cell surface membrane pump hydrogen ions (protons) from the cytoplasm into the cell wall and intercellular spaces. This creates a higher concentration of protons outside the companion cell than inside. Protons cannot diffuse through the membrane down this concentration gradient back into the cell because they are charged. The gradient provides the energy for moving sucrose into the companion cell. Carrier proteins in the cell surface membrane accept both sucrose and protons on the external surface of the membrane. Each carrier protein changes shape when a proton and a sucrose molecule are both attached and transfers them into the cytoplasm. These carrier proteins are co-transporters as they transport two substances at the same time. Sucrose then diffuses through plasmodesmata into sieve tubes. Companion cells have many mitochondria to provide ATP for proton pumping and have plasmodesmata for diffusion of sucrose into sieve tubes.

(iv) Xylem vessel elements transport xylem sap, which consists mostly of water and ions. They are elongated cells joined end to end; they have no end walls and no contents so there is little resistance to the flow of sap. The cells have thick cellulose cell walls impregnated with lignin that acts as a waterproofing and provides thickening so the cells do not collapse inwards.

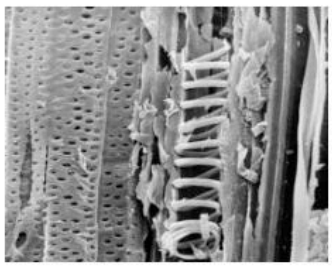
(b) The source is a plant organ that assimilates substances, such as sucrose and amino acids, and loads them into the phloem sieve tubes. Leaves are an example. The sink is the site of unloading of substances from phloem and needs them to provide energy and materials for growth (e.g. in protein synthesis) or for storage. Roots are an example.

(c) Phloem transports assimilates, such as sucrose and amino acids. These are not transported by xylem, which transports water and ions instead. Phloem sap travels up and down the stems of plants, whereas water only flows upwards in the xylem. Phloem sap moves by pressure flow and xylem sap by cohesion-tension.

Raise your grade

1 The scanning electron micrograph shows some xylem vessels.

(a) Explain how features of xylem vessels, **visible in the SEM**, are adaptations for the movement of water over long distances. [4]



The xylem vessels have thick cell walls to withstand the inward force on walls due to the adhesion between water molecules and cellulose cell walls. ✔ The xylem vessels have no end walls ✔ and no cell contents to reduce resistance to flow of water. ✔ Vessels also have pits to allow water to travel laterally to other cells, e.g. in the stem. The spiral thickening gives strength to a xylem vessel but also allows it to stretch when the plant grows in length. ✔

Correct answers

(b) Students used a potometer to measure the rate of water uptake of leafy shoots of croton, *Codiaeum variegatum*, at different times during the day so that they could investigate the effect of temperature. The table shows their results.

Experiment	Temperature/°C	Wind speed (setting on fan)	Mean rate of movement of gas bubble/mm h^{-1}
1	15	low	12
2	15	high	22
3	25	low	24
4	25	high	45
5	35	low	64
6	35	high	120

Using the data in the table, describe *and* explain the effects of the two conditions that the students changed on the rate of water uptake. [6]

description

With an increase in temperature from 15°C to 35°C there was an increase in the rate of uptake of water, ✔ e.g. from 15°C to 25°C at low speed, the rate increased from 12 to 24 mm h^{-1}. ✔ With an increase in wind speed there is an increase in the rate of uptake of water ✔, e.g. at 35°C, the rate increases from 64 to 120 mm h^{-1} ✔ when wind speed increases.

explanation

When the temperature increases molecules have more kinetic energy ✔ so rates of evaporation and diffusion increase so more molecules of water vapour pass out through stomata. ✔ When there is an increase in wind speed more water vapour is removed from the air just outside stomata, therefore a steeper water potential gradient between air inside leaf and the atmosphere. ✔

This answer covers all aspects of the question. Note how the candidate has written two sub-headings. This is good practice for questions that have two command words – here 'describe' *and* 'explain'.

2 Aphids and spittlebugs (froghoppers) are small insects that have mouthparts adapted for sucking liquids. Aphids feed on phloem sap and spittlebugs feed on xylem sap.

(a) Suggest why aphids are likely to show faster growth rates than spittlebugs. [3]

Aphids feed on phloem sap which contains sucrose and amino acids. ✔ The sucrose provides energy to the aphids and the amino acids are used to make proteins so the aphids can grow. ✔

The right idea, but the candidate has not explained why spittlebugs do not grow as fast. To gain full marks the answer needs to include that xylem contains very few organic compounds to provide energy or materials for making proteins, carbohydrates, fats and nucleic acids.

Ringing (also known as girdling) is used to investigate the movement of solutes in stems. A complete ring of tissue external to the xylem is removed.

The concentrations of sucrose were determined in several parts of a stem that were ringed. Samples were also taken from the same positions on the stem of an unringed control plant. The results are shown in the table.

Part of the plant where phloem sap sample taken	Concentration of sucrose/arbitrary units	
	Ringed plant	Unringed (control) plant
in the stem above the ring	0.60	0.43
in the stem below the ring	0.00	0.41
in the roots	0.03	0.30

(b) (i) With reference to the table, describe the effect of ringing. [3]

All the sucrose remains above the ring rather than moving downwards. ✘

Incorrect answer. The question implies that answers should make use of the results either by quoting them or carrying out one or more calculations with the data. The candidate could have stated that there is no sucrose below the ring, whereas in the control the concentration was 0.41 au.

(ii) Explain the effect of ringing on the distribution of sucrose. [2]

Ringing removes the phloem tissue ✔ so sucrose does not move from the leaves (source) to the roots (sink). ✔ This is why there is a high concentration above the ring.

Correct answer that explains why there is no sucrose below the ring

Exam-style questions

1 Where is the Casparian strip located in a plant?

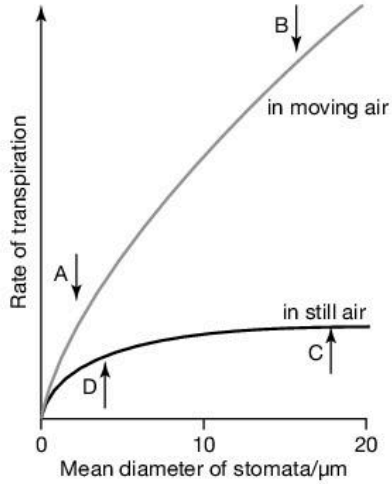
A endodermis in roots

B epidermis in leaves

C xylem in stems

D phloem in leaves [1]

2 The graph shows the rate of transpiration of *Tradescantia zebrina* in still air and in moving air.



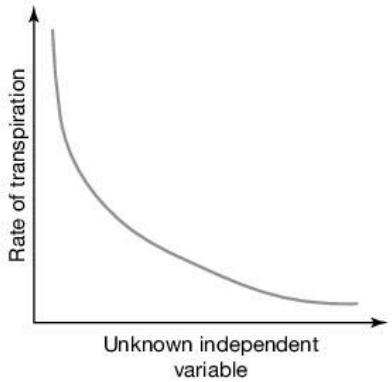
Which letter indicates the mean stomatal diameter that is not limiting the rate of transpiration? [1]

3 The table shows the water potentials of four plant tissues.

Tissue	Water potential/MPa
A	–0.01
B	–1.07
C	–2.60
D	–3.67

Which tissue will show very little change in mass when immersed in distilled water? [1]

4 The graph shows the effect of an environmental factor on the rate of transpiration.



Which is the independent variable?

A humidity

B light intensity

C temperature

D wind speed [1]

5 Companion cells in phloem tissue have carrier molecules that act as co-transporters.

Which describes the action of this co-transporter mechanism?

A moving glucose into the companion cell

B moving hydrogen out of the companion cell

C moving sodium out of the companion cell

D moving sucrose into the companion cell [1]

6 A student investigated the rate of transpiration. The mass of water lost over 24 hours was 620 g. The total surface area of the leaves was estimated as 16 m^2.

Which shows the rate of transpiration?

A $0.8\,g\,m^{-2}\,h^{-1}$

B $1.6\,g\,m^{-2}\,h^{-1}$

C $26\,g\,m^{-2}\,h^{-1}$

D $39\,g\,m^{-2}\,h^{-1}$ [1]

7 (a) Flowering plants are multicellular. Explain why they need a transport system. [3]

(b) A molecule of sucrose moves from a mesophyll cell in a leaf to a cell in the root tuber of a sweet potato plant.

Explain the mechanisms involved in the movement of sucrose

(i) from a mesophyll cell in a leaf into a companion cell. [4]

(ii) from a sieve tube in a leaf to the root tuber. [5]

8 Transport in mammals

Key points

- ☐ State that the mammalian circulatory system is a closed double circulation consisting of heart, blood vessels and blood.
- ☐ Recognise arteries, veins and capillaries in prepared slides and in photomicrographs and electron micrographs.
- ☐ Make plan diagrams of the structure of arteries, veins and capillaries using prepared slides.
- ☐ Explain the relationship between the structure and function of arteries, veins and capillaries.
- ☐ Draw the structure of red blood cells, monocytes, neutrophils and lymphocytes using prepared slides and photomicrographs.
- ☐ State and explain the differences between blood, tissue fluid and lymph.
- ☐ Describe the role of haemoglobin in carrying oxygen and carbon dioxide with reference to carbonic anhydrase, haemoglobinic acid and carbaminohaemoglobin.
- ☐ Describe and explain the significance of the oxygen dissociation curves of adult haemoglobin at different carbon dioxide concentrations (the Bohr effect).
- ☐ Describe and explain the significance of the higher red blood cell count of humans at high altitude.
- ☐ Describe the external and internal structure of the mammalian heart and explain the differences in the thickness of the walls of the different chambers in terms of their functions with reference to resistance to flow.
- ☐ Describe the cardiac cycle and explain how heart action is initiated and controlled.

The circulatory system of mammals

The circulatory system of a mammal is a **closed double circulation** consisting of the heart, blood vessels and the blood.

The heart is the pump that keeps the blood flowing through the circulation.

The double circulation has two circuits:

- **pulmonary circulation** – blood flows from the heart to the lungs in the pulmonary arteries and returns to the heart in the pulmonary veins;
- **systemic circulation** – blood is pumped by the heart into the aorta and then through arteries to all the organs, except the lungs; blood returns to the heart in veins which empty into the vena cava, which is the body's main vein leading into the heart.

The advantage of a double circulation is that blood is sent to different parts of the body at different pressures. Blood flows through the lungs at a much lower pressure than that in the systemic circulation, which prevents damage to the delicate capillaries in the lungs. A high pressure in the aorta means that blood is delivered to other organs at high pressures so there is an efficient supply of oxygen and nutrients.

★ Exam tip

Find a diagram that shows the circulatory system of a mammal. Follow the pathway of a blood cell as it travels through organs such as the liver, intestines and kidney. This will help you to explain what is meant by a *closed double circulation*.

Key terms

Closed circulation: blood flows around the body within vessels.

Double circulation: blood flows through the heart twice in one complete circulation of the body.

★ Exam tip

Think of the heart as a double pump: pumping blood through the pulmonary circulation for gas exchange in the lungs and pumping blood through the systemic circulation to supply all the other organs with oxygen, nutrients, hormones and to remove waste substances, such as urea and carbon dioxide. Do not confuse a double pump with a double circulation.

Blood vessels

▼ **Table 8.1** The functions of the three main types of blood vessel in a mammal

Blood vessel	Function
artery	• carries blood **away from the heart** at high pressure • stretches and recoils to maintain the blood pressure • delivers blood to organs at a pressure slightly less than it left the heart
vein	• carries blood **towards the heart** at low pressure • expands to take increasing volumes of blood, e.g. during exercise • as blood pressure is low, backflow of blood is prevented by semi-lunar valves at intervals
capillary	• carries blood **between arteries and veins** at low pressure and low speed • allows the exchange of respiratory gases, solutes and water between blood and tissue fluid

Link

The movement of blood in blood vessels is another example of mass flow (see Unit 7).

Practical skills

Exam tip

Use a calibrated eyepiece graticule so your drawings have the same proportions as the structures you are studying. In Paper 3, you may have to take measurements of the blood vessels and calculate magnifications of your drawings.

Looking at blood vessels

You should look at microscope slides and images of the three types of blood vessels and make plan diagrams to show the tissues within their walls.

Capillaries are best viewed as photomicrographs and electron micrographs (see Figures 8.5 and 8.6).

The walls of arteries and veins have three regions:

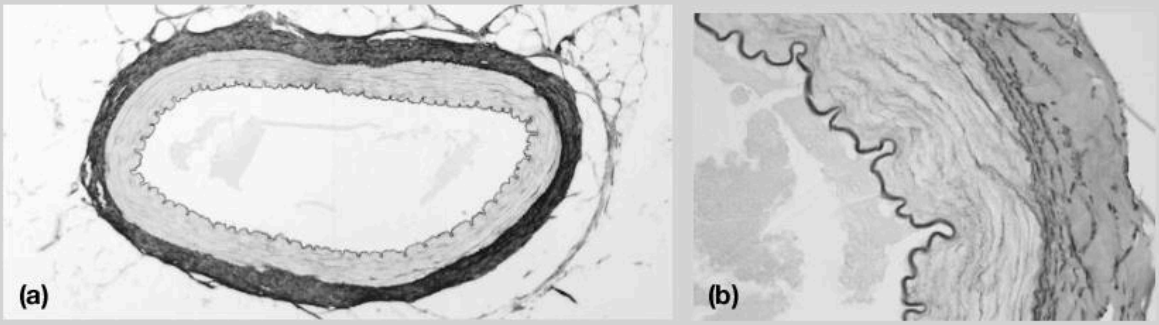
Tunica intima (also known as tunica interna) is a single layer of endothelial cells and some elastic tissue. In images of arteries, the tunica intima often has a corrugated (or crinkly) appearance which is a result of the loss of blood pressure during the preparation of microscope slides. When filled with blood, the walls of arteries are round in cross section and smooth, and the elastic tissue is stretched.

Tunica media is formed of smooth muscle tissue, elastic fibres and collagen fibres. In arteries this is the thickest of the three regions.

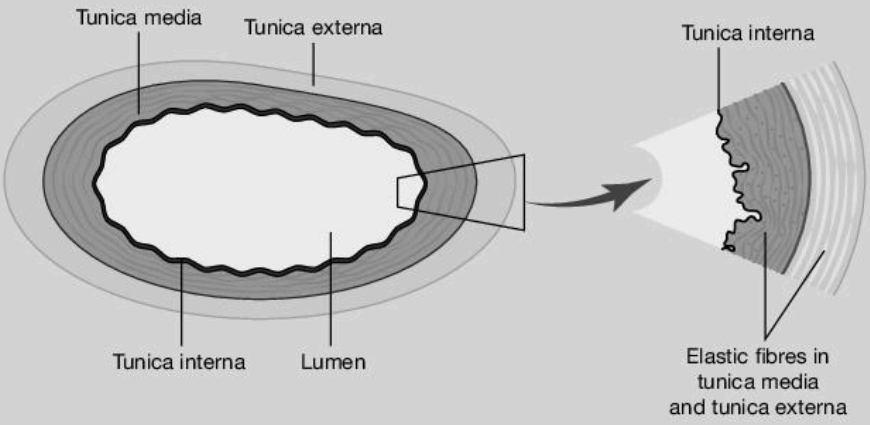
Tunica adventitia (tunica externa) is the outer region composed mostly of collagen fibres with some elastic fibres.

Structure of arteries

There are two types of artery: elastic artery and muscular artery. Elastic arteries, such as the aorta, are close to the heart and muscular arteries are further away delivering blood into organs.



▲ **Figure 8.1** Photomicrographs of **(a)** a cross-section of a muscular artery at low power ×12 **(b)** detail of the wall at higher power ×40



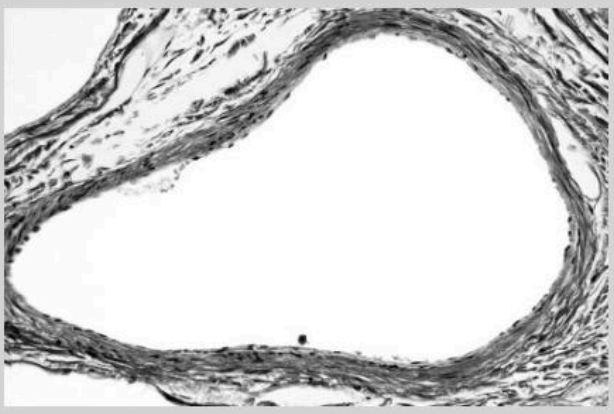
▲ **Figure 8.2** Drawings of the muscular artery in Figure 8.1

Exam tip

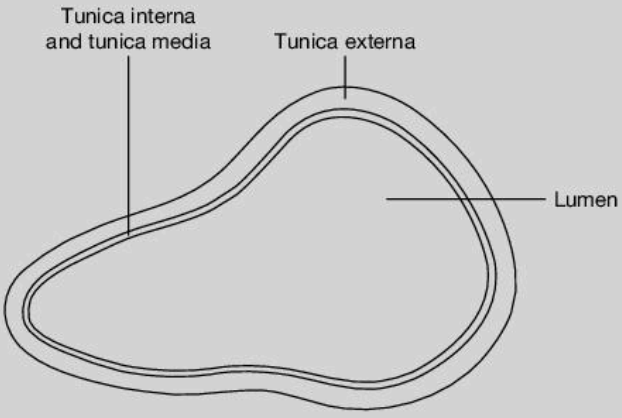
If you are asked to add annotations to a plan diagram, you should use them to describe what you can see. This could include the relative proportions of the layers and details of the staining or the appearance of the tissues. Do not shade your diagram and draw the lines with a sharp pencil.

Structure of veins

The blood pressure in veins is much less than in arteries so the walls are much thinner as there is far less smooth muscle and elastic tissue. Notice that veins rarely have regular shapes when prepared for the microscope, so make sure that you draw the shape that you see as carefully as possible.



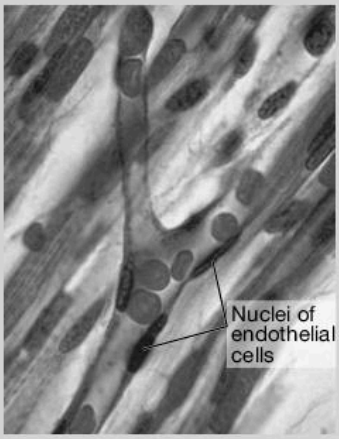
▲ **Figure 8.3** A cross-section of a vein at low power ×150



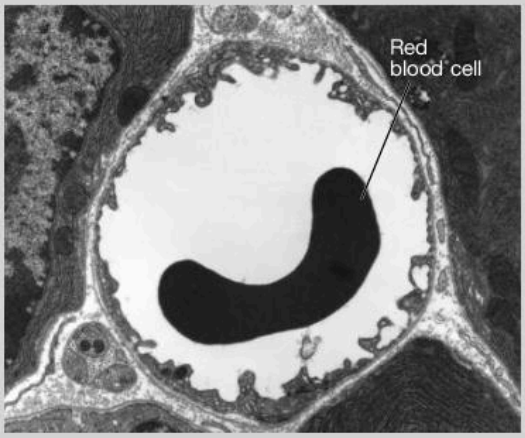
▲ **Figure 8.4** A plan diagram of the vein in Figure 8.3

Structure of capillaries

The blood pressure in capillaries is low so that the delicate walls made of a single layer of endothelium are not damaged. The pressure at the arterial end is sufficiently high to cause water and solutes to pass from the blood into tissue fluid. The movement of water and solutes such as glucose and amino acids is made easier by the small pores between the endothelial cells. Oxygen and carbon dioxide pass through these pores, but can diffuse through the endothelial cells.



▲ **Figure 8.5** A photomicrograph showing red blood cells moving in single file inside a capillary. ×550



▲ **Figure 8.6** TEM of a cross-section of a capillary. The diameter is about the same as that of a red blood cell. ×6000

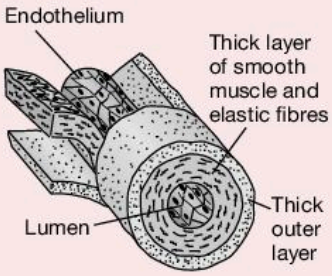
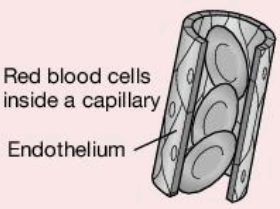
Exam tip

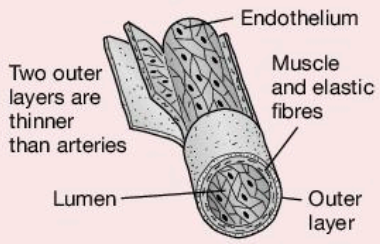
Capillary walls are formed of endothelial cells; it is not correct to say that they have cell walls, as that is confusing them with plants.

Exam tip

You are expected to be able to make a drawing of a capillary and it is best to practise drawing one from a TEM such as the one in Figure 8.6. See Unit 20 Practical assessment page 242 for details about how to do this.

▼ **Table 8.2** The structural features of the three main types of blood vessel and the relationship between structure and function

Blood vessel	Structural features	Relationship between structure and function
artery 	endothelium	smooth inner lining to reduce chances of turbulent flow which damages endothelium and promotes blood clotting
	high ratio of wall thickness to diameter of lumen	
	thick layer of elastic tissue	elastic tissue stretches as blood is pumped into an artery; it recoils to maintain pressure
	thick layer smooth muscle	smooth muscle maintains a tension in the artery to help maintain blood pressure
	thick outer layer of collagen fibres	collagen fibres give strength to prevent bursting due to high blood pressure
capillary 	only an endothelium, no other cells or fibres	short diffusion distance for exchange of substances
		thin vessels so no cell is far from a capillary
	pores between the endothelial cells	perforated with pores to allow water and solutes to pass out into tissue fluid by pressure filtration

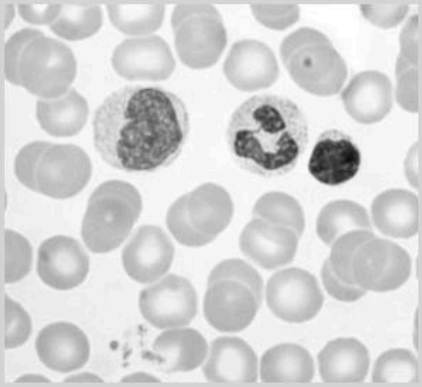
Blood vessel	Structural features	Relationship between structure and function
vein 	endothelium low ratio of wall thickness to lumen diameter little elastic tissue and smooth muscle thin outer layer of collagen fibres valves	smooth lining (see above) diameter of vein can increase to take more blood, e.g. during exercise blood pressure is low so less of these are present prevent backflow of blood

Practical skills

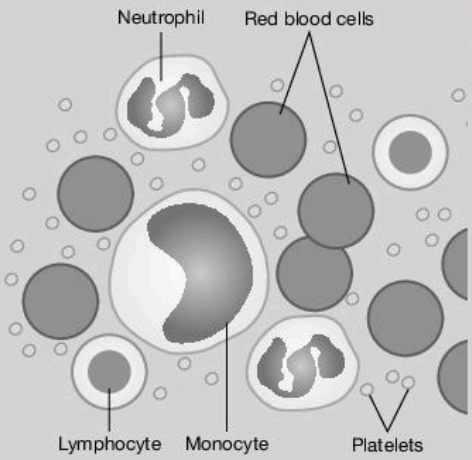
Blood cells

Take a prepared slide of mammalian blood and look carefully under low power to find the faint red or pink layer. Focus carefully, turn to high power and refocus. Take care to keep the objective lens above the cover slip while you search and focus under high power (Figure 8.7).

The nuclei of white blood cells are often stained blue. Find the three different types of white blood cell listed in Table 8.3 by searching across the slide. Red blood cells have no nuclei and are usually pink, often with an almost clear centre.



▲ **Figure 8.7** Blood cells photographed using the high power of a microscope. The nucleated cells are (from left to right) a monocyte, a neutrophil and a lymphocyte (×800)



▲ **Figure 8.8** The types of blood cells shown in Figure 8.7. You should be able to recognise these in slides of blood and in photographs

Exam tip

White blood cells vary in size. If you are asked to identify these cells from a photo or a drawing, always look at the shape of their nuclei rather than their size.

Blood, tissue fluid and lymph

All exchanges between blood and cells occur through **tissue fluid**. These include:

- oxygen diffusing out of the blood into tissue fluid
- carbon dioxide diffusing from tissue fluid into the blood
- water and some solutes forced into tissue fluid by the pressure of the blood.

Water and substances are forced out by high pressure from the blood by **pressure filtration**. As blood flows through the capillaries its pressure decreases, which makes it possible for water to pass back into the blood plasma by osmosis. Blood contains solutes, such as albumen, which give the blood plasma a lower water potential than the tissue fluid. Albumen is a large protein molecule that cannot easily leave the blood through the capillary walls.

Key term

Tissue fluid: the extracellular fluid around all the cells of the body. It is formed by pressure filtration from the blood and removed by reabsorption into the blood and by drainage into the lymphatic system.

▼ **Table 8.3** The features and functions of the major blood cells

Type of blood cell	Features	Functions
red blood cell (erythrocyte)	• small cell (7 µm diameter) • shape: biconcave disc • flexible membrane • no nucleus; no organelles (mitochondria, RER, Golgi body) • cytoplasm is full of millions of molecules of haemoglobin • contains enzyme carbonic anhydrase	can change shape and fit easily through capillaries more space to fill with haemoglobin to transport oxygen and carbon dioxide catalyses reactions to help transport carbon dioxide
neutrophil	• large cell (10 µm diameter) • lobed nucleus • small nucleus: cytoplasm ratio • mitochondria, RER, Golgi body • many lysosomes containing hydrolases	lobed nucleus helps cells leave blood through spaces between endothelial cells in capillary walls protein synthesis to make hydrolase enzymes for intracellular digestion of bacteria and other pathogens
monocyte	• large cell (10–20 µm diameter) • kidney bean-shaped nucleus • mitochondria, RER, Golgi body	these cells are in the blood travelling to tissues where they become macrophages, which are long-lived phagocytic cells
lymphocyte (B-lymphocytes and T-lymphocytes)	• small cell (4–6 µm diameter) • large nucleus to cytoplasm ratio • highly specific cell surface receptors	B-lymphocytes are activated to become plasma cells which secrete antibodies; T-lymphocytes have a variety of roles (see Unit 11)

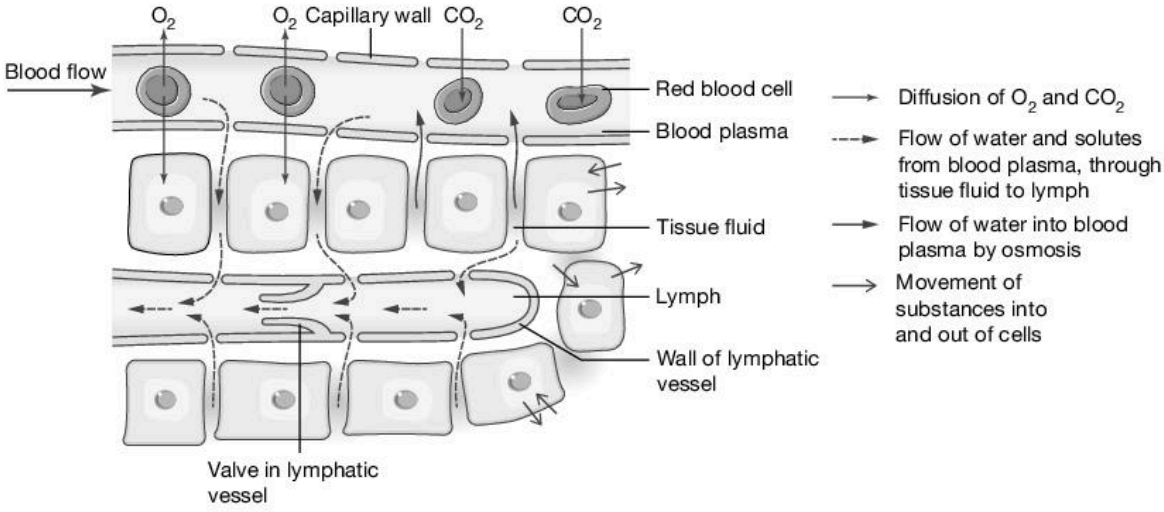
▼ **Table 8.4** The composition and functions of three body fluids

Feature	Blood	Tissue fluid	Lymph
where found	in blood vessels	surrounding cells	in lymphatic capillaries and vessels
components:			
• red blood cells	✔	✘	✘
• white blood cells	✔	✔ (some)	✔
• fats	✔ (as lipoproteins)	✔	✔ (especially after a meal)
• glucose	✔	✔	very little
• proteins	✔ (e.g. antibodies and albumen)	✔ (some, e.g. antibodies)	✔ (some, e.g. antibodies)
functions	transport	surrounds cells – all exchanges between blood and cells occur through tissue fluid	drains excess tissue fluid, preventing a build up leading to oedema (swelling of tissues)

Within the tissues are lymphatic capillaries, which are small, thin walled, blind-ending tubes (see Figure 8.9). Tissue fluid enters the lymphatic capillaries and then flows quite slowly towards larger lymphatic vessels that empty into the blood near the heart. The fluid inside lymphatic capillaries and lymphatic vessels is called **lymph** and it is similar in composition to tissue fluid.

Key term

Lymph: a fluid found within lymphatic vessels. It is formed from tissue fluid and is returned to the blood.



▲ **Figure 8.9** The relationship between three body fluids: blood, tissue fluid and lymph. Blood plasma is the liquid part of the blood

Haemoglobin and the transport of oxygen

Haemoglobin is a conjugated protein with quaternary structure. The four parts of the molecule are two α-globin polypeptides and two β-globin polypeptides. Each is associated with a haem group. Oxygen combines loosely with the iron in the haem group. When this happens, the whole molecule changes shape making it easier for haemoglobin to accept more oxygen. Each haemoglobin molecule can transport four molecules of oxygen to form oxyhaemoglobin:

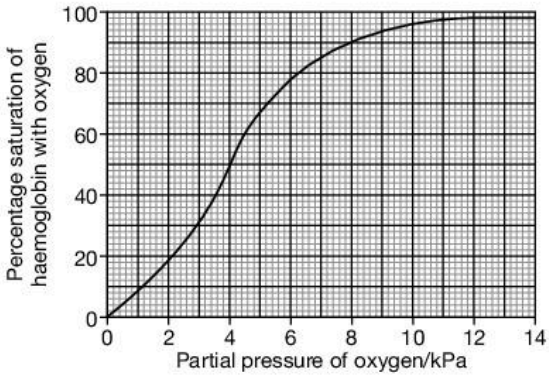
$$Hb + 4O_2 \rightarrow HbO_8$$

The binding of one molecule of oxygen to haemoglobin makes it easier to bind another; once the second one has bound, it makes it easier to bind the third and so on. This **cooperative binding** is responsible for the results obtained from investigations into the ability of haemoglobin to take up and supply oxygen.

Partial pressure is the pressure exerted by one gas as part of a gas mixture. The concentration of oxygen in different parts of the body can be equated with partial pressures in the atmosphere. The concentrations of oxygen in tissues are much lower as the oxygen is being used in respiration.

Tissues	Partial pressure of oxygen (pO_2)/kPa
lungs	13.3
resting muscle	5.0
active muscle, e.g. during strenuous exercise	3.5

◀ **Table 8.5** Partial pressures of oxygen in mammalian tissues



◀ **Figure 8.10** An oxygen haemoglobin dissociation curve. Think of the *x*-axis as the 'availability of oxygen' and the *y*-axis as the 'affinity of haemoglobin for oxygen'

You need to know how changes in the availability of oxygen influence the oxygen-carrying capacity of haemoglobin in the blood.

Remember

It is a good idea to revise the structure of haemoglobin from Unit 2.

Link

See Unit 9 for details of gas exchange in the lungs.

Exam tip

Make a copy of the oxygen haemoglobin dissociation curve on a piece of graph paper. You can annotate your graph to show which part is equivalent to loading in the lungs (about 13.3kPa) and which part is equivalent to unloading in the tissues (5.0–3.5kPa). Use it to practise using this graph to read off percentage saturations at specific partial pressures of oxygen.

Figure 8.10 is an oxygen haemoglobin *dissociation* curve because you start at the right with loading of blood with oxygen in the lungs, and move to the left with unloading of oxygen from the blood in the tissues. From right to left, oxygen *dissociates* from haemoglobin. The graph shows the results of an experiment to investigate the response of haemoglobin to different concentrations of oxygen. The steep part of the line coincides with the partial pressures of oxygen in respiring tissues where oxyhaemoglobin dissociates to provide them with oxygen (5.0 kPa down to about 3.5 kPa). A slight decrease in the pO_2 in the tissues stimulates much dissociation, so oxyhaemoglobin gives its oxygen to the tissues.

Haemoglobin and the transport of carbon dioxide

Carbon dioxide diffuses into blood plasma from respiring cells. It is highly soluble in water and about 5% of carbon dioxide transported in the blood dissolves in the plasma. Some also reacts with water in the plasma to form hydrogen carbonate ions, but this is a slow reaction as it is not catalysed by an enzyme. Most of the carbon dioxide diffuses down its concentration gradient into red blood cells.

About 10% of the carbon dioxide in the blood enters red blood cells and combines with the $-NH_2$ terminals of the polypeptides that make up haemoglobin to form **carbaminohaemoglobin**.

Red blood cells contain the enzyme carbonic anhydrase which catalyses the following reaction to form carbonic acid which then dissociates:

$$CO_2 + H_2O \xrightarrow{\text{carbonic anhydrase}} H_2CO_3 \rightarrow H^+ + HCO_3^-$$

- Hydrogen carbonate ions – these ions accumulate inside the cytoplasm of the red blood cells as they travel along capillaries in respiring tissues. Their concentration is greater than that in the plasma so they diffuse out of the cells through specialised channel proteins into the plasma. About 85% of the carbon dioxide transported in the blood is carried as hydrogencarbonate ions in the plasma, where they associate with sodium ions and form part of the buffer system that maintains the blood at a constant pH.
- Hydrogen ions – to prevent accumulation of these ions in red cells – which would lower the pH and decrease the activity of enzymes – haemoglobin acts as a buffer, absorbing the hydrogen ions to maintain a constant pH. This lowers the affinity of haemoglobin for oxygen and promotes the dissociation of oxyhaemoglobin. This is key to understanding the effect that carbon dioxide has on the unloading of oxygen from oxyhaemoglobin in respiring tissues. When the rate of respiration increases – as it does in muscle tissue during exercise – there are more hydrogen ions produced in the red cells, more are absorbed by haemoglobin so more oxygen is released. The increase in carbon dioxide in the blood stimulates:
  - the release of *more* oxygen, which allows
  - *more* aerobic respiration to occur.

  This extra dissociation reduces the reliance on anaerobic respiration, which would lead to production of lactate and eventually to fatigue.

Unloading carbon dioxide from the blood

When the blood reaches the lungs, all the events described above go into reverse. There is a low concentration of carbon dioxide in the alveoli so some carbon dioxide starts diffusing out of the blood. Also, there is a high

Exam tip

Do not confuse **carbamino**haemoglobin which forms with **carbon dioxide** with **carboxy**haemoglobin which forms with **carbon monoxide** (see Unit 9).

Exam tip

Carbonic anhydrase is one of the fastest acting enzymes. Its turnover number is 600 000 s^{-1} which means it can process 600 000 molecules of carbon dioxide in one second.

Exam tip

Look carefully at the oxygen dissociation curves in Question 5 on page 102 and re-read the paragraphs about loading blood with carbon dioxide. Notice where the word *more* is used. Oxyhaemoglobin unloads oxygen in tissues because of the low pO_2, but if the pCO_2 in the tissues increases, this stimulates it to unload even more. Use the word 'more' when explaining this effect.

concentration of oxygen in the alveoli and this diffuses into the red blood cells. At high concentrations of oxygen, haemoglobin has a higher affinity for oxygen than hydrogen ions, so these leave and provide a substrate for the reaction catalysed by carbonic anhydrase:

$$H^+ + HCO_3^- \xrightarrow{\text{carbonic anhydrase}} H_2CO_3 \rightarrow H_2O + CO_2$$

The carbon dioxide diffuses down its concentration gradient out of the red cells and into the alveoli.

The effect of carbon dioxide on the saturation of haemoglobin with oxygen can be seen in the graph on page 102. The curve is shifted to the right. This effect is known as the **Bohr effect**. Use a ruler to study this graph more carefully. You can see that the result of increasing the pCO_2 from 2.7 to 10.7 kPa is to shift the curve to the right. But the important point about this is the difference between the saturation at *each* partial pressure of oxygen. To see this, put a ruler at a partial pressure of oxygen corresponding to that in the tissues. You could choose any partial pressure between 3 and 5 kPa on the *x*-axis. Put your ruler vertically and read off the values from the *y*-axis. The saturation with oxygen is *less* when carbon dioxide is present. This means that oxyhaemoglobin unloads *more* oxygen than when the pCO_2 is less.

Exam tip

Carbon dioxide must pass through five cell surface membranes to move from red blood cells to the alveoli.

The effect of high altitude on blood

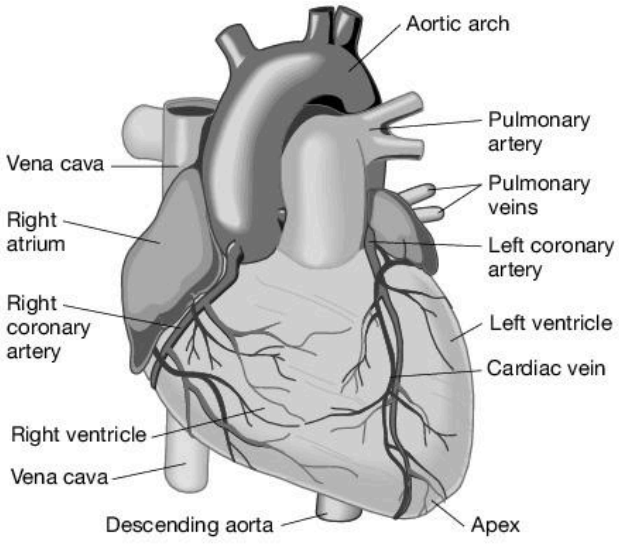
At high altitude there is a lower partial pressure of oxygen. Human haemoglobin has a lower affinity for oxygen at high altitude. People who live permanently at high altitude have a higher proportion of red blood cells than people who live at lower altitudes (e.g. at sea level). People who move up to high altitude or who fly to places like La Paz in Bolivia (4000 m above sea level), find that they are short of breath. The air pressure is much lower than at sea level and although 20% of the air is still oxygen, the partial pressure is much less. To compensate for this, more red blood cells are made and the percentage by volume increases from 45% of blood volume to as much as 70%. This **acclimatisation** takes about a week and is stimulated by the secretion of a hormone known by its abbreviation as EPO.

Link

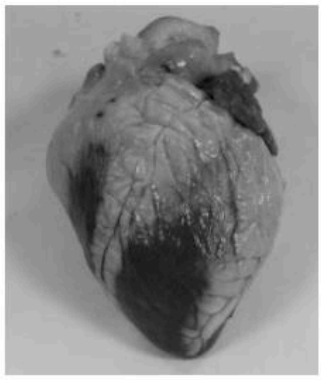
Raise your Grade Question 2 on page 101 has some data on the blood of two people living at different altitudes.

The heart

Figures 8.11 show the external structure of a mammalian heart.

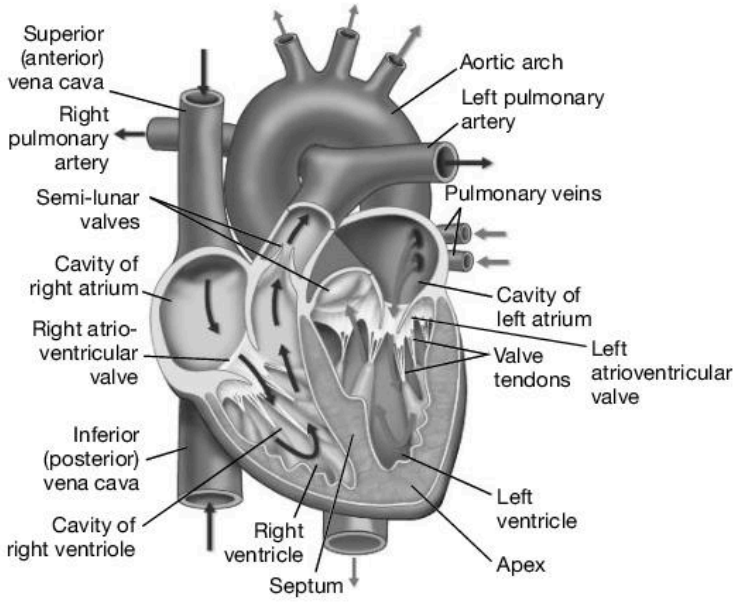


▲ **Figure 8.11** The external structure of the heart showing the main blood vessels and the heart's own blood supply



▲ **Figure 8.12** The outside of the heart as seen from the ventral surface. This is the same view of the heart as in Figure 8.11

The heart consists of two pumps working in series. Deoxygenated blood flows into the right side of the heart, which pumps it into the pulmonary circulation; oxygenated blood returns from the lungs to the left side of the heart where it is pumped into the systemic circulation. The two sides contract and relax together at the same time.



▲ **Figure 8.13** The internal structure of the heart showing the four valves. Black arrows show flow of deoxygenated blood; grey arrows show flow of oxygenated blood

▲ **Figure 8.14** A heart dissected to show the internal structures of the left side. You can see the left atrium, bicuspid valve, left ventricle, valve tendons and the papillary muscles at the base of the tendons

A complete and a dissected heart do not look exactly like those in Figures 8.11 and 8.13. It is a good idea to dissect a heart, or watch one being dissected, so you can see what the structures look like and to appreciate how the heart works.

Watch an animation of the heart showing what happens as it beats. You should see the events of **systole**, the contraction phase, and **diastole**, the relaxation phase.

The heart does not pump out a fixed volume of blood all the time. During exercise, more blood returns in the veins per minute than at rest. The heart responds by expanding to a greater volume during diastole and then pumping out the increased volume of blood per beat.

Key terms

Systole: the phase of the cardiac cycle when contraction occurs to increase blood pressure. The atrial systole occurs before ventricular.

Diastole: the relaxation phase of the cardiac cycle.

Worked example

The volume of blood pumped out by each ventricle is the **stroke volume**. This is between 60 and 80 cm^3 at rest increasing to 200 cm^3 during strenuous exercise. The **cardiac output** is the volume of blood pumped out by the left ventricle per minute.

a) A person has a stroke volume at rest of 70 cm^3. The heart rate is 75 beats per minute. Calculate the cardiac output.

b) The cardiac output for a man doing strenuous exercise is 25 $dm^3\ min^{-1}$.

Calculate the stroke volume if the heart rate is 120 beats min^{-1}.

c) Suggest why (i) the volumes of blood ejected by the left and right ventricles are always the same at any one time, and (ii) these volumes increase during exercise.

Answers

a) Cardiac output = $(70 \times 75) = 5.3\ dm^3\ min^{-1}$ (2 s.f.)

b) Stroke volume = $(25/120) = 210\ cm^3$ (2 s.f.)

c) (i) The volume of blood pumped out by the right ventricle passes via the pulmonary circulation to the left ventricle. The same volume must be pumped by both ventricles at the same time otherwise some blood will remain in the lungs.

(ii) The total volume of oxygen delivered to the organs, especially the muscles, needs to increase to maintain aerobic respiration that supplies energy for muscle contraction. More blood is pumped by the heart to the lungs to be oxygenated and more is pumped to the rest of the body to supply oxygen. There is also increased delivery of glucose and increased removal of carbon dioxide from respiring tissues.

Control of the heart

Every time the heart beats, the two atria contract together and almost immediately afterwards, the ventricles contract together forcing blood into the arteries. These actions of the heart muscle need to be coordinated.

Coordination of the muscles in your arms and legs is achieved entirely by the nervous system (see Unit 15 pages 171 to 176). Cardiac muscle is different from skeletal muscle because it is **myogenic**. There is a system of specialised cardiac muscle cells that initiate (start) the heart beat by emitting impulses that spread across cardiac muscle so it contracts in a coordinated way. It ensures that the ventricles contract after the atria and that the ventricles contract from the base upwards. The specialised cardiac muscle that coordinates the heat beat consists of:

- **sinoatrial node** (SAN) situated in the muscle of the right atrium
- **atrioventricular node** (AVN) situated between the atria and the ventricles
- **Purkyne fibres** that run down towards the apex of the heart within the muscular wall between the ventricles and into the ventricular muscle.

The SAN sends out impulses that travel across the cardiac muscle in the atria. Cardiac muscle consists of interconnected cells so that the impulse spreads across the muscle in all directions. The impulse stimulates cardiac muscle in the atria to contract. The impulse cannot pass directly from the atria to the ventricles because there is a ring of non-conducting fibrous tissue separating the two. The impulse reaches the AVN where it is slowed for 0.1 s before passing to the Purkyne fibres. These fibres conduct the impulse down the septum to the base of the ventricles and then to the rest of the muscle in the walls of the ventricles so they contract from the bottom upwards. The AVN therefore relays the impulse from the SAN to the ventricles, but after a short delay.

The heart rate is not entirely dependent on the rate at which the SAN sends out impulses. The activity of the SAN is influenced by the nervous system and by the hormone adrenaline.

The cardiac cycle

Trace the pathway taken by blood through the heart and the pulmonary and systemic circulations by using Figure 8.13. Do not confuse the pathway taken by blood with the cardiac cycle, which is the changes that occur within the heart during one heartbeat.

The biggest changes in pressure occur on the left side of the heart so it is usually the left that is used to show the cardiac cycle.

The graph in Figure 8.17 shows changes in blood pressure in the left side of the heart during one heartbeat. Follow the changes by putting a ruler vertically against the *y*-axis and moving it to the right.

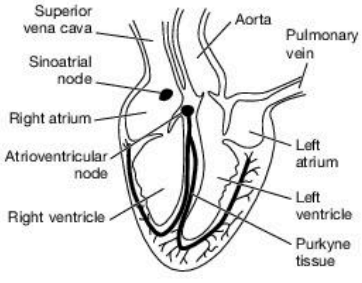
Key terms

Myogenic: a contraction is myogenic if it is started within a muscle and not stimulated by impulses from nerves that terminate on muscle.

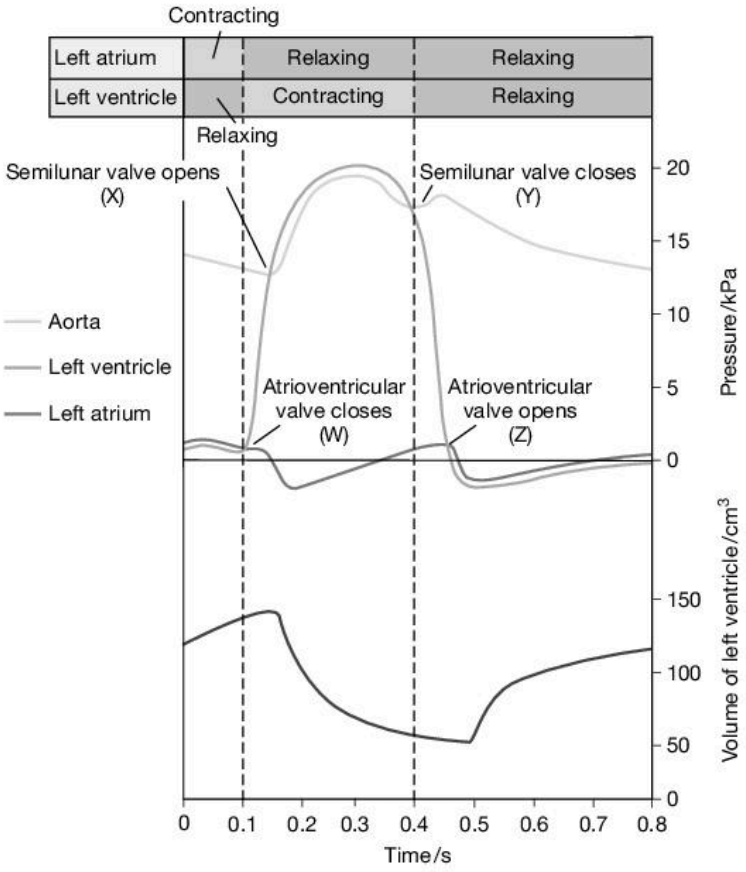
Sinoatrial node: part of the cardiac muscle in the right atrium that sends out impulses to cause cardiac muscle to contract. Often called the heart's 'pacemaker'.

Atrioventricular node: part of the cardiac muscle situated between the atria and the ventricles that relays impulses from the SAN to the Purkyne fibres to cause contraction of the ventricles.

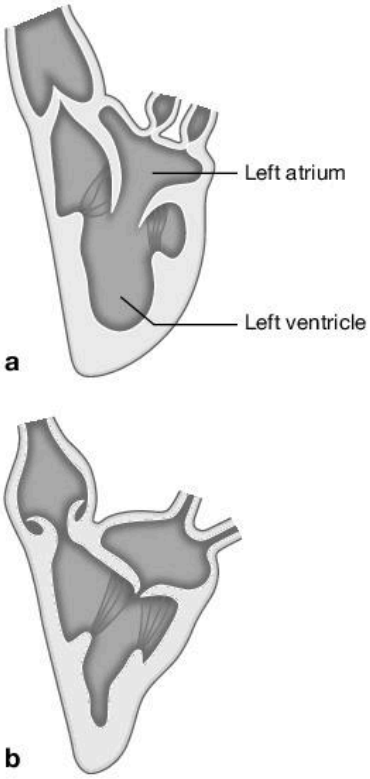
Purkyne fibres: specialised cardiac muscle fibres that transmit impulses to the muscles of the ventricle walls.



▲ **Figure 8.15** The positions of the SAN, AVN and Purkyne fibres in the heart (pulmonary artery not shown)



▲ **Figure 8.17** The pressure changes in the left side of the heart and in the aorta during one cardiac cycle



▲ **Figure 8.16** The left side of the heart at two different stages of the cardiac cycle: **a** atrial systole and **b** ventricular systole. Compare with Figure 8.17

The graph shows that at **W**, **X**, **Y** and **Z** the pressures in the left atrium and the left ventricle change with respect to each other.

At **W**, the pressure in the left ventricle increases above that in the left atrium. This forces the atrioventricular valve to close. The blood moves under the 'flaps' of the valve and the valve tendons prevent the valve 'blowing back' into the left atrium.

At **X**, the pressure in the left ventricle increases so that it is above that of the aorta. This causes the semi-lunar valves to open so that blood flows into the aorta.

At **Y**, the left ventricle is relaxing and expanding. The blood pressure decreases so that it is lower than that of blood in the aorta. The blood from the aorta fills the 'pockets' of the semi-lunar valves and they close.

At **Z**, the blood pressure of the ventricle decreases to below that of blood in the left atrium so the atrioventricular valve opens.

Exam tip

You should be able to interpret this graph. Try searching online for 'cardiac cycle animation' to find something to help you follow the changes in the heart. Notice how the volume of the left ventricle changes during the cycle. The volume decreases during systole and increases during diastole.

Exam tip

The impulses emitted by the SAN that pass across the heart are also called waves of excitation. Either term can be used, but do not use 'messages' or 'signals' as these are both too vague.

Exam tip

'The AVN delays and relays' – a way to remember the role of this part of the heart.

Raise your grade

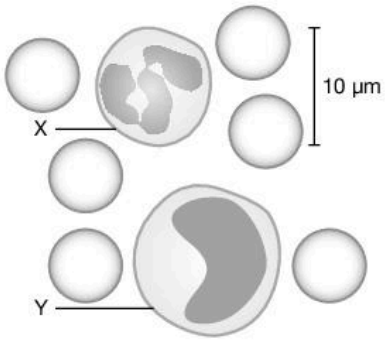
1 (a) Mammals have a closed, double circulation.

Explain how the structure of the heart enables it to pump blood into two circulations at different pressures. [4]

The heart is divided into two halves, separated by the septum ✔ so that deoxygenated blood and oxygenated blood are kept separate. ✔ The pressure of blood leaving the heart is caused by the thickness of the cardiac muscle. The thicker the muscle the greater the pressure. The left ventricle has a thicker wall ✔ and pumps blood at a much higher pressure into the systemic circulation than the right ventricle ✔, which pumps blood into the pulmonary circulation.

Correct answer. The candidate has answered each aspect of the circulation given in the question. It was a good idea to start by explaining the role of the septum.

(b) The drawing was made from a photomicrograph of mammalian blood. The drawing shows two white blood cells and some red blood cells.



(i) Calculate the magnification of the drawing. Show your working and express your answer to the nearest whole number. [2]

The length of the scale bar is 1.5cm. This = 1500µm ✘. The magnification = 1500 / 10 = x 150 ✘

The candidate measured the length of cell **X** scale bar in centimetres and incorrectly multiplied by 1000 to give micrometres (there are 10000µm in a cm). It is much better to measure in millimetres, not in centimetres. The actual magnification = x1500.

(ii) State the names of the cells labelled X and Y and give a reason for each of your answers. [4]

The cells are white blood cells and they protect the body against infections. They have nuclei unlike the surrounding red blood cells.

The candidate seems to have misread the question. There are two cells to be named: Cell **X** is a neutrophil and Cell **Y** is a monocyte. Reasons: their nuclei have characteristic shapes: **X** has a lobed nucleus, **Y** has a kidney bean-shaped nucleus.

2 (a) Blood plasma and lymph are two body fluids.

Explain how lymph is formed from blood plasma. [3]

Blood plasma is filtered as it passes through capillaries. ✔ All the filtered substances form the fluid in between the cells in the tissue. ✔ To stop the tissue fluid building up some of it flows into capillaries and it forms lymph. ✘

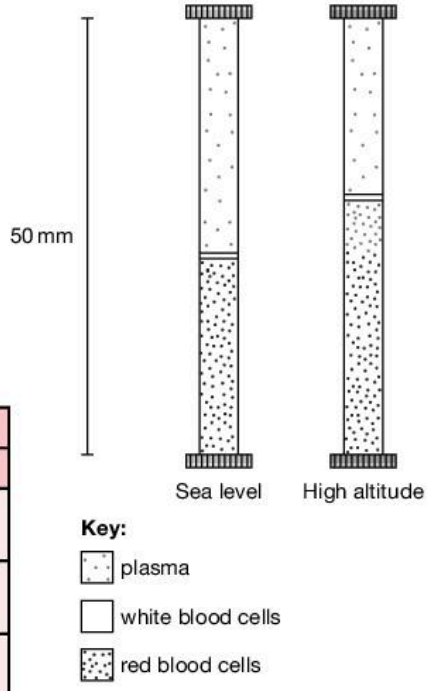
The candidate has made a good start; it would be better to name the process as *pressure filtration*. The answer needs to distinguish between *blood* capillaries and *lymphatic* capillaries, as tissue fluid flows into both. Reread your answers before you finish the exam paper.

(b) Samples of blood were taken from two Peruvians. One of them lived at sea level and the other at high altitude (5000 m). Three measurements were made:

- the number of red blood cells in each sample
- the percentage of the total volume of the blood occupied by red blood cells (the haematocrit) as shown in the diagram
- the concentration of haemoglobin.

The results are shown in the table.

	Blood samples from Peruvians at:	
	Sea level	**High altitude**
number of red blood cells /cells mm^{-3}	5.0×10^6	6.4×10^6
haematocrit/%	45.0	
concentration of haemoglobin/mg 100 mm^{-3}	15.0	20.0



(i) Calculate the haematocrit for the sample of blood taken from the person who lives at 5000 m. [1]

60% ✔

Correct answer. The candidate has measured the height of the tube that contains the red blood cells, divided by the total height of the blood sample and multiplied by 100.

(ii) Use the information in the table to explain how the haemoglobin concentration is increased in people living at high altitude. [2]

The haemoglobin concentration is increased by increasing the number of red blood cells by 28% so that they occupy a greater volume of the blood. ✔ The volume of the plasma has decreased (from 55% to 40%) so the concentration of red blood cells has increased. ✔

The candidate has followed the clue given in part (i) by calculating the percentage increase in red blood cells and the percentage decrease of the plasma. This is a much better way to answer a question like this than simply quoting the figures from the table.

Exam-style questions

1 The following are found in the walls of blood vessels.

1 collagen fibres

2 elastic fibres

3 endothelial cells

4 smooth muscle cells

Which are found in the walls of veins?

A 1, 2, 3 and 4

B 1 and 2 only

C 3 and 4 only

D 1 and 4 only [1]

2 During which stage in the cardiac cycle do semi-lunar valves at the base of the pulmonary artery open?

A atrial diastole

B atrial systole

C ventricular diastole

D ventricular systole [1]

3 Which describes the enzyme carbonic anhydrase?

	Site of action	Substrate	Product(s)
A	blood plasma	carbon dioxide and water	hydrogen carbonate ions
B	blood plasma	haemoglobin and oxygen	oxyhaemoglobin
C	cytoplasm of red blood cells	carbon dioxide and water	carbonic acid
D	cytoplasm of red blood cells	haemoglobin and carbon dioxide	carbaminohaemo-globin

[1]

4 Which part of the circulation contains oxygenated blood?

A coronary veins

B right atrium

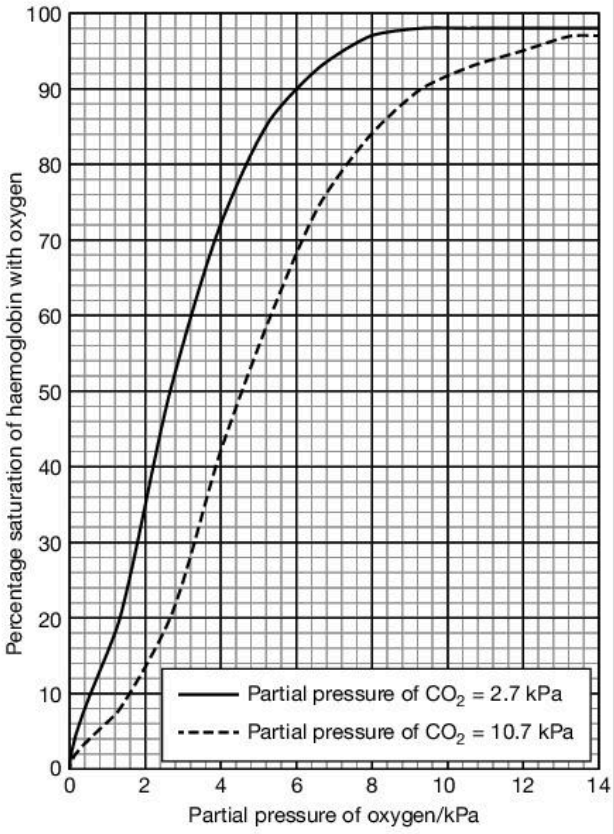
C pulmonary veins

D vena cava [1]

5 One of the functions of blood is to transport carbon dioxide.

(a) Outline what happens to carbon dioxide when it enters the blood from respiring cells so that it can be transported to the lungs. [5]

The graph shows the effect of two partial pressures of carbon dioxide on the oxygen dissociation curve for haemoglobin.



(b) State the percentage saturation of haemoglobin with oxygen at a pO_2 of 4.0 kPa at the two partial pressures of carbon dioxide, 2.7 kPa and 10.7 kPa. [1]

(c) (i) State the name of the effect of increasing pCO_2 on the saturation of haemoglobin with oxygen. [1]

(ii) Explain how the effect you have named in (i) ensures an efficient delivery of oxygen to tissues during exercise. [4]

extra questions available online

9 Gas exchange and smoking

Key points

- ☐ Describe the gross structure of the human gas exchange system.
- ☐ Draw plan diagrams of the structure of the walls of the trachea, bronchi, bronchioles and alveoli.
- ☐ State the distribution of cartilage, ciliated epithelium, goblet cells, smooth muscle, squamous epithelium and blood vessels.
- ☐ Describe the functions of cartilage, cilia, goblet cells, mucous glands, smooth muscle and elastic fibres.
- ☐ Recognise the cells and tissues of the gas exchange system in prepared microscope slides, photomicrographs and electron micrographs.
- ☐ Describe the process of gas exchange between air in the alveoli and the blood.
- ☐ Describe the effects of tar (and the carcinogens it contains) in tobacco smoke on the gas exchange system with reference to lung cancer and chronic pulmonary obstructive disease (COPD).
- ☐ Describe the short-term effects of nicotine and carbon monoxide on the cardiovascular system.

The human gas exchange system

You should be able to locate the organs that comprise the gas exchange system. Check you can do this by using diagrams from text books and web sites. The distribution and functions of the cells and tissues that compose the system are described in Table 9.1.

The trachea, the two bronchi entering the lungs and the bronchioles form the airways of the gas exchange system. The trachea branches to form two bronchi. Bronchi have a similar structure to the trachea, but the cartilage is in blocks in the walls of the bronchi instead of in rings as it is in the trachea. Bronchioles have no cartilage.

Exam tip

In this Unit, you do not need to know how the breathing movements ventilate the lungs.

▼ **Table 9.1** Components of the gas exchange system and their functions

Structure	Distribution in gas exchange system	Functions
ciliated epithelium	trachea, bronchi, bronchioles	cilia move mucus up the airways
goblet cells	trachea, bronchi, larger bronchioles	secrete mucus onto the surface of the ciliated epithelium
mucous glands	trachea, bronchi	secrete mucus into ducts that open through the ciliated epithelium
cartilage	trachea, bronchi	holds open the airways to allow easy flow of air
smooth muscle	trachea, bronchus and bronchioles	contracts to narrow the airways
elastic fibres	in all parts of the system especially the alveoli	stretch when breathing in; recoil when breathing out helping to force air out of the lungs
squamous epithelium	alveoli	thin to give a short diffusion pathway for gas exchange; alveoli provide a large surface area
capillaries	in all parts of the system – many around the alveoli	provide large surface for exchange between blood and air in the alveoli

Exam tip

You should be clear about three different aspects of the gas exchange system:

- **ventilation** — breathing air in and out of the lungs
- **gas exchange** — diffusion of oxygen and carbon dioxide between air in the alveoli and the blood.

Exam tip

Do not confuse breathing and gas exchange with respiration, which is the chemical processes that occur inside cells to transfer energy from molecules, such as glucose and fat, to ATP.

The airways allow the uninterrupted flow of air into and out of the lungs. To do this they must be kept open and be able to respond to demands for an increased oxygen supply by widening when necessary.

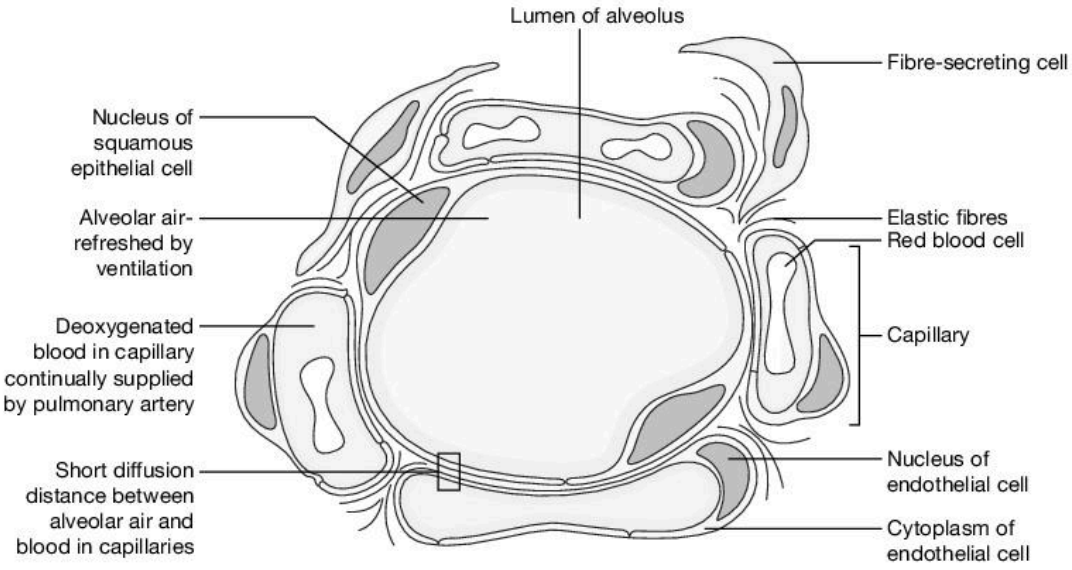
The airways also protect the gas exchange surface. The lining of the airways is formed by ciliated epithelium that contains goblet cells. Beneath the epithelium there are mucous glands. Goblet cells and mucous glands secrete mucus onto the surface of the epithelium to trap any small particles – including pathogens, such as bacteria, viruses and fungal spores – that have passed the hairs in the nose.

Link

See Unit 10 for the different types of pathogen and the ways in which they are transmitted from person to person. The pathogen that causes TB is transmitted through the air in droplets of water.

Gas exchange in the alveolus

Alveoli are tiny air-filled sacs, adapted for the efficient exchange of gases by diffusion between the air and blood capillaries. This requires a **short diffusion distance** (less than 0.3 μm), a **large surface area** and a **steep concentration gradient**.



▲ **Figure 9.1** Features of an alveolus – the gas exchange surface in the lungs

Breathing ventilates the alveoli, maintaining near constant concentrations of oxygen and carbon dioxide in alveolar air. Blood flows through capillaries in the lungs, bringing a constant supply of deoxygenated blood. Ventilation and blood flow combine to maintain a steep concentration gradient for oxygen between the blood and alveolar air. The same is also true for carbon dioxide, although the concentration gradient is not as steep as it is for oxygen.

Remember

In the lungs, oxygen diffuses into the blood, carbon dioxide diffuses out of the blood.

Smoking and disease

The components of tobacco smoke have short-term and long-term effects on the health of the gas exchange and cardiovascular systems. Make sure that you know the difference between the two systems.

The important constituents of cigarette smoke are **tar** (a thick, black, oily liquid that contains carcinogens and settles in the bronchi and bronchioles), **carbon monoxide** (a gas that combines with haemoglobin) and **nicotine** (the drug in tobacco that is absorbed into the blood).

Carcinogens are cancer-causing chemicals, e.g. benzpyrene.

Exam tip

If you see a question on smoking do not automatically think it is asking about the effects on the lungs (gas exchange system), it may be asking about the short-term effects on the cardiovascular system.

The effects of tar on the gas exchange system

Tar is an irritant, causing inflammation of the epithelium lining the tract. The goblet cells and mucous glands secrete more mucus which accumulates in the bronchi, the cilia are inhibited so mucus is not moved upwards resulting in a suitable environment for pathogens to grow. Long-term smoking causes progressive changes in the linings of the airways, particularly in the bronchi, leading to **chronic bronchitis**. Smoking stimulates macrophages in the alveoli, which in turn release chemicals that attract neutrophils. Proteases, particularly elastase, are secreted by these neutrophils and macrophages. Proteases are enzymes that digest lung tissue and are responsible for the damage seen in **emphysema**.

Chronic obstructive pulmonary disease (**COPD**) is a collective term used for conditions that occur in the bronchi and lungs including chronic bronchitis and emphysema. Smoking is the major cause.

Emphysema often leads to heart failure.

Many cases of **lung cancer** are related to smoking. The carcinogens in tobacco smoke interact with DNA in bronchial epithelial cells causing mutations. Cells become tumerous if the genes that control the cell cycle and mitosis mutate.

The short-term effects of nicotine and carbon monoxide on the cardiovascular system

Carbon monoxide diffuses into red blood cells where it combines irreversibly with haemoglobin to form **carboxyhaemoglobin** so less haemoglobin is available to take up oxygen. Nicotine is rapidly absorbed and acts at synapses, stimulating the nervous system to the heart and promoting the secretion of adrenaline from the adrenal glands. This increases heart rate and blood pressure. Nicotine also stimulates neurones that supply arterioles. The neurones stimulate smooth muscle to contract so reducing blood flow to capillaries.

Both carbon monoxide and nicotine can damage the endothelium that lines blood vessels. This damage leads to inflammation increasing the risks of:

- atheroma – build-up of fatty deposits in the walls of arteries, especially the coronary arteries
- thrombosis – formation of blood clots.

Link

The gas exchange system is covered in this Unit, the cardiovascular system was covered in Unit 8.

Key terms

Chronic bronchitis: long-term disease involving the inflammation of the walls of the bronchi and other air passages so that breathing becomes difficult.

Emphysema: lung disease in which the walls of alveoli are destroyed. This leads to the formation of larger air sacs and a decrease in the surface area for gas exchange, making breathing difficult.

Chronic obstructive pulmonary disease: the long-term lung diseases chronic bronchitis and emphysema.

Exam tip

The word chronic means long-term, although many think it means severe or very harmful. The word acute is used for short-term diseases.

Exam tip

Chronic bronchitis often leads to emphysema.

Key term

Carboxyhaemoglobin: the irreversible combination of carbon monoxide with haemoglobin.

Practical skills

Making plan diagrams of the trachea and lungs

Study prepared slides of the trachea and the lungs. In sections of a lung look for sections through a bronchus, bronchioles, alveoli and capillaries. Using the high power of your microscope find all the structures listed in Table 9.1.

You should be able to make plan diagrams of:

- the trachea, to show the distribution of the tissues
- the lungs, to show bronchi, bronchioles, branches of the pulmonary artery and vein, and the alveoli.

You must be able to recognise the different cells and tissues. Always draw what you see, not what you remember drawing or seeing in a book.

Make drawings of representative areas in the wall of the trachea or bronchus seen with the high power of your microscope. Recognise and identify the different cell types and describe their appearance, for example, the colours that they are stained.

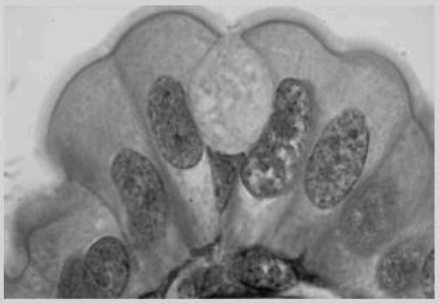
Exam tip

When making these plan diagrams, you should not draw any cells, as explained in Unit 20 Practical assessment page 242.

Exam tip

Make a list of tissues and cells that you are expected to recognise and write a description of each one. Search online for web sites that have images of human tissues and organs. You could start with 'histology guide Leeds University' although there are many to choose from.

▲ **Figure 9.2** A photomicrograph of a cross section of the trachea of a small mammal



▲ **Figure 9.3** A photomicrograph showing a goblet cell from a ciliated epithelium of the bronchus

Worked example

This question is based on questions in Paper 3.

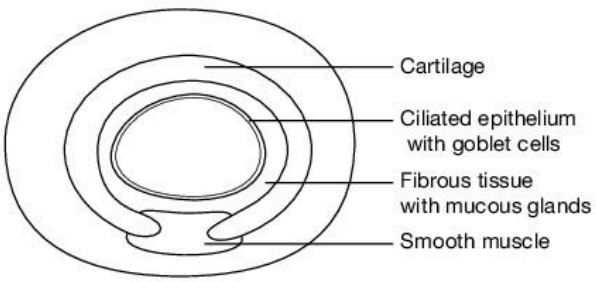
a) Make a plan diagram of the cross-section of the photomicrograph of the trachea in Figure 9.2. Label the tissues shown in your drawing.

b) The actual distance across the lumen of the trachea is 3.5 mm. Calculate the magnification of your plan diagram and add this information to your drawing. Show your working.

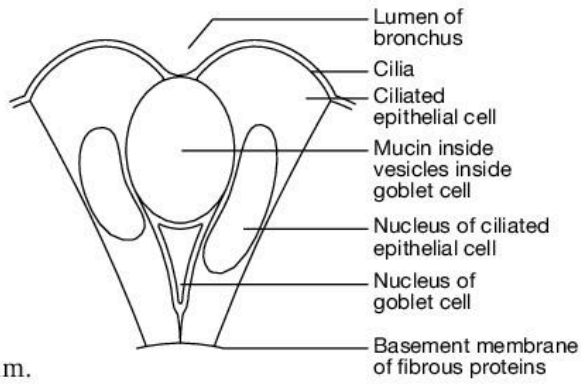
c) Make a drawing of the goblet cell and the two adjacent cells visible in Figure 9.3. Show on your answer to **(a)** the region of the trachea where goblet cells are found.

Answers

a)



c)



b) Distance across the trachea in the plan diagram is 17 mm.
Magnification = 17 ÷ 35 = ×4.9

Raise your grade

1 (a) (i) Describe the structure of the tissue that forms the lining of the bronchus. [2]

The lining is made from a ciliated epithelium. ✔ This has many cells with cilia facing into the lumen. In between these cells are goblet cells that make mucus and release it onto the surface. ✔ The mucus is sticky and any small particles in the air attach to it.

Correct answer that starts well naming the tissue and stating that goblet cells are present. This is enough to gain full marks. The function of mucus is not relevant to the question so the extra detail does not gain marks.

(ii) Explain how the distribution of cartilage in the lining of the bronchus differs from the distribution in the trachea. [2]

The cartilage in the trachea forms rings that are arranged at intervals down the length of the organ. There are no rings in the bronchus, just pieces of cartilage not organised into rings. ✔

The candidate correctly identified the main difference between the trachea and the bronchus. However, the rings are not described as C-shaped or incomplete rings.

(b) During exercise, changes occur in the bronchi and in the arteries that supply leg muscles. Outline the changes that occur in both of these structures. [6]

The trachea widens during exercise so that more air can reach the lungs. More blood flows in an artery during exercise. This is because the muscle contracts much more to move the blood.

The candidate has answered the question about the trachea rather than the bronchi. Even though the principle is the same, no marks are awarded. 'More air' and 'more blood' should be more precise. The candidate could refer to a greater volume per minute or to a great speed of flow. Blood is pumped into arteries by the ventricles. The smooth muscle in arteries does *not* contract to move blood along. No marks are awarded for the answer.

2 (a) Complete the passage about lung cancer by using the most appropriate word (or words).

Cigarette smoke contains carcogens ✘ that stimulate changes in DNA. These changes are known as mutations ✔ and they increase the chances that cells in the lining of the bronchus will start to divide uncontrollably by mitosis ✔. A cell that starts to divide in this way may form a group of cells known as a tumor ✔. These cells may invade healthy lung tissue and block the airways or blood vessels. One of the symptoms of lung cancer is coughing up blood ✔ [5]

The candidate has spelt 'carcinogens' incorrectly. 'Tumor' is the American spelling so would be accepted. It is important to spell words correctly although American spellings of words, such as hemoglobin and tumor, are accepted.

A long-term study of the effects of smoking on health was undertaken in the USA.

A large group of people was divided into two groups. One group consisted of smokers, the control group consisted of non-smokers. When these people died their causes of death were recorded.

The table shows the results of the study.

Cause of death	Deaths among smokers (A)	Deaths among non-smokers (B)	Excess of deaths (A – B)	Percentage of excess (A – B/total of excess deaths)	Relative death rate (A/B)
CHD	3361	1973	1388	52.1	1.70
stroke	556	428	128	4.8	1.30
lung cancer	397	37	360	13.5	10.73
cancer of mouth and oesophagus	91	18	73	2.7	5.06
other causes	2911	2195	716	26.9	1.33
total	7316	4651	2665	100.0	1.57

For each cause of death in the table:

- the number of excess deaths is calculated as the difference between the number of deaths among the smokers and the number among the non-smokers
- the contribution of each cause to the total of excess deaths is given as the percentage of the excess
- the relative death rate is calculated as the number of deaths in the non-smoker group divided by the number among the smokers. This is a measure of the risk of smoking leading to each of the causes of death in the table.

(b) Suggest two precautions that should be taken when choosing the non-smokers for the control group in a study of this type. [2]

They should all be the same age,✔ same gender,✔

Correct answers. In questions like this, make sure that you give the number of answers required.

(c) A student who analysed these results concluded that 'smoking causes more deaths from cardiovascular disease than lung cancer'.

Explain whether the data in the table support this statement or not. [4]

No. I would not agree. The data shows that smoking increases the risk of getting lung cancer by ten times, but among the people studied 360 more people died from lung cancer than might have been expected to die from that disease.✔ For CHD the risk is 1.7 (stroke = 1.30) which means there is a much lower risk,✔ but 1516 people died of CVD (CHD + stroke) than might have been expected if smoking had no effect.✔ This was 56.9% of the 'excess' deaths.✔

The candidate has analysed the data thoroughly and understands the concept of risk. The answer also shows that the candidate knows that stroke and CHD are both cardiovascular diseases (CVDs). The candidate has also said that the statement is wrong when in fact it is correct. The examiner may set questions in which there is evidence that supports the statement and also some that does not. Be prepared to agree with the statement or disagree, but you must give evidence in support of your answer.

Exam-style questions

1 The gas exchange system contains these cells and tissues:

1 cartilage

2 ciliated epithelium

3 elastic fibres

4 goblet cells

Which are found in the wall of the trachea?

A 1, 2, 3 and 4; **B** 1 and 3 only;

C 2, 3 and 4 only; **D** 2 and 4 only [1]

2 Which contains all the components of tobacco smoke that cross the gas exchange surface?

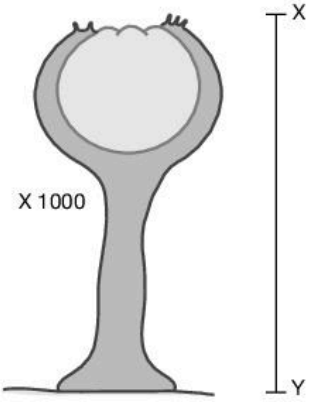
A carbon dioxide and tar

B carbon monoxide and tar

C carbon dioxide and nicotine

D carbon monoxide and nicotine [1]

3 The drawing was made from an electron micrograph of a goblet cell.



What is the length of the goblet cell from **X** to **Y**?

A 5.0 mm **B** 5.0 µm **C** 50.0 µm **D** 500.0 nm [1]

4 Which feature of the mammalian gas exchange surface is an adaptation for reducing the diffusion distance between air and blood?

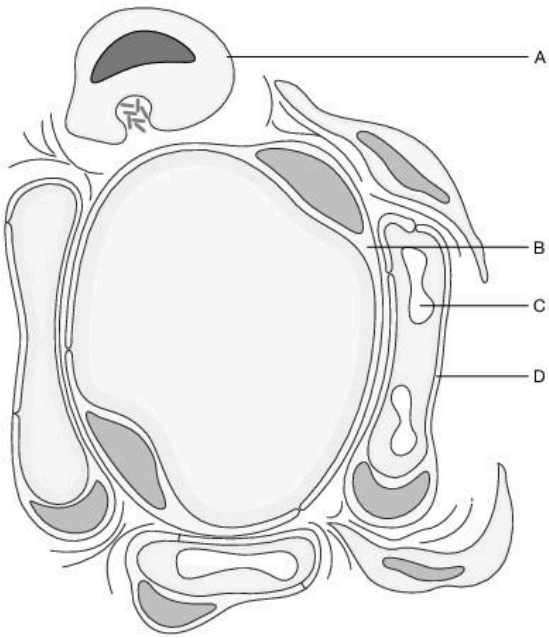
A many capillaries

B moist surface

C elastic fibres

D squamous epithelial cells [1]

5 **(a)** Gas exchange in mammals occurs in the alveoli. The drawing shows an alveolus and surrounding structures.



The table shows some details of four cells that are labelled in the drawing. Complete the table by:

- naming the cells
- stating the function of each cell
- stating the letter that identifies each cell

Name of cell	Function of cell	Letter on drawing
	phagocytosis	
		B
red blood cell		
	forms the wall of the capillary	

[4]

(b) State four structural features of the trachea that are adaptations for its function. Explain how each feature is an adaptation. [4]

extra questions available online

10 Infectious disease

Key points

- ☐ Define the term disease.
- ☐ Explain the difference between an infectious disease and a non-infectious disease (limited to sickle cell anaemia and lung cancer).
- ☐ State the name and type of causative organism (pathogen) of each of the following diseases: cholera, malaria, tuberculosis (TB), HIV/AIDS, smallpox and measles.
- ☐ Explain how cholera, measles, malaria, TB and HIV/AIDS are transmitted.
- ☐ Discuss the biological, social and economic factors that need to be considered in the prevention and control of cholera, measles, malaria, TB and HIV/AIDS.
- ☐ Discuss the factors that influence the global patterns of distribution of malaria, TB and HIV/AIDS.
- ☐ Outline how penicillin acts on bacteria.
- ☐ Explain why antibiotics do not affect viruses.
- ☐ Explain how bacteria become resistant to antibiotics with reference to mutation and selection.
- ☐ Discuss the consequences of antibiotic resistance.
- ☐ Describe steps that can be taken to reduce the impact of antibiotic resistance.

AS Level

Diseases

Disease is often defined as a malfunction of the mind or body. A disease can be due to a single cause or have several causes (multifactorial). A disease can affect a specific tissue or organ or affect many parts of the body. A simple way to classify human diseases is to divide them into two groups:

- **infectious diseases** are caused by organisms that invade the body, and use components of host cells for their growth and/or replication and cause harm
- **non-infectious diseases** have other causes; examples are inherited diseases (e.g. cystic fibrosis and sickle cell anaemia) and diseases caused by poor diet (e.g. scurvy and rickets).

Infectious and non-infectious diseases

Infectious diseases are caused by a disease-causing organism or **pathogen**. Most human pathogens are microorganisms belonging to three main types. In order of size, these are:

- viruses
- bacteria
- protoctists.

Viruses are non-cellular. Bacteria are prokaryotic organisms. **Protoctists** are eukaryotic organisms. Table 10.1 lists the six infectious diseases that you need to know about.

Non-infectious diseases are all the other categories of disease that are not caused by a pathogen. The two examples that you should know are:

- sickle cell anaemia – an inherited disease caused by a mutant allele (see Unit 6 and Unit 16 pages 185 and 189), and
- lung cancer – a degenerative disease which, left untreated, becomes progressively worse (see Unit 9 page 105).

Key terms

Disease: disorder or illness, associated with poor functioning of part of the body or mind.

Infectious disease: any disease that is caused by a pathogen.

Non-infectious disease: any disease that is not caused by a pathogen.

Pathogen: any disease-causing organism that lives in or on the body of a host.

Protoctist: any eukaryotic organism that is not classified as a fungus, plant, or animal. Some protoctists are unicellular (e.g. *Plasmodium*) and others are multicellular.

Link

There is more about protoctists in Unit 17.

▼ **Table 10.1** The causative organisms of six infectious diseases

Disease	Causative organism (pathogen)	
	Type	**Name**
malaria	protoctist	several species of *Plasmodium*, e.g. *P. falciparum, P. malariae, P. ovale, P. vivax*
cholera	bacterium	*Vibrio cholerae*
Tuberculosis (TB)	bacterium	*Mycobacterium tuberculosis* *Mycobacterium bovis*
HIV/AIDS	virus	Human Immunodeficiency Virus (HIV)
measles	virus	*Morbillivirus*
smallpox	virus	*Variola*

Table 10.1 shows you how to write the scientific names of the six pathogens that you must know. In print, these names are always given in italics. You can underline them if you wish when you write them but it is not essential. You must use a capital letter for the first or only name and a lower case letter for the second name. If you use a name such as *Plasmodium falciparum* more than once then you can abbreviate the first name to the initial letter with a full stop, as in Table 10.1.

Exam tip

The names of the causative organisms in Table 10.1 must be spelt correctly in exam answers.

Remember

This is a good opportunity to revise the similarities and differences between prokaryotes and eukaryotes. See Unit 1 pages 5–6. Exam questions often cover two or more units, such as Units 1 and 10.

Transmission refers to the way in which a pathogen is transferred from an **infected host** to an **uninfected host**. This is also known as the **transmission cycle** as it continues from host to host. Some pathogens only have one method of transmission, others have several methods. Read Table 10.2 carefully and you will be able to identify the various factors that increase the risk of being infected by each pathogen. These factors are not only biological, but also aspects of where people live and their living conditions. The factors that put most people at risk of infectious diseases are poverty and poor housing.

Exam tip

Look carefully at the heading of Table 10.2. It is the pathogens that cause infectious diseases which are transmitted between uninfected and infected people.

▼ **Table 10.2** The main methods of transmission of five pathogens

Disease	Main methods of transmission of pathogen
cholera	**faecal-oral route** *V. cholerae* passed out in faeces of infected people contaminate drinking water and food; uninfected people consume water and food contaminated with the bacteria
malaria	**insect vector** – female *Anopheles* mosquito mosquito takes a blood meal from an infected person; the blood contains reproductive stages of *Plasmodium*; the mosquito then takes another blood meal from an uninfected person, passing infective stages in its saliva into that person's blood when it injects anticoagulant
TB	**airborne droplets of water** infected people breathe or sneeze out TB bacteria *(M. tuberculosis)* in droplets of water; uninfected people breathe in these airborne droplets *M. bovis* is also transmitted in unpasteurised milk and meat from infected cattle
HIV/AIDS	**body fluid contact** • HIV transmitted in semen during sexual intercourse (both vaginal sex and anal sex) • blood containing HIV taken from infected person and passed to uninfected person by e.g. intravenous drug abusers sharing hypodermic needles, also in blood used for transfusion and blood products • HIV crosses the placenta from mother to fetus and in breast milk to baby
measles	**airborne droplets of water** infected people breathe or sneeze out *Morbillivirus* particles in droplets of water and mucus; uninfected people breathe in these droplets

★ Exam tip

Malaria is caused by several species of *Plasmodium*, e.g. *P. falciparum*. The pathogens that cause malaria are transmitted by mosquitoes. Malaria is *not* caused by mosquitoes.

Worked example

A risk factor is any factor that increases the chance that a person will develop a disease.

a) Explain why cholera is categorised as an infectious disease, but lung cancer is not.

b) (i) State **two** risk factors associated with cholera.

(ii) Explain what is meant by the term *transmission* as applied to infectious diseases.

Answers

a) Infectious diseases, such as cholera, are caused by pathogens that enter the body. Non-infectious diseases are not caused by pathogens. The cause of many cases of lung cancer is tobacco smoking.

b) (i) No or very poor sanitation; drinking water contaminated by human faeces.

(ii) The movement of a pathogen from an infected person to a non-infected person.

The prevention and control of infectious diseases

Disease prevention uses methods that stop the transmission of pathogens to uninfected people. These methods prevent the spread of infectious diseases by breaking the transmission cycle. Vaccination is a successful method of prevention that has been used to eradicate smallpox (see Unit 11 page 126 to discover how this was done).

Disease control uses methods that reduce the number of people who are infected, for example, during an epidemic. This also involves breaking the transmission cycle but concentrates on reducing the transfer of pathogens from people who are infected, for example, using insecticides to kill insect vectors, such as *Anopheles*.

▼ **Table 10.3** Prevention and control methods for cholera

Prevention and control methods for cholera	Problems with implementing prevention and control methods
provide good sanitation to remove and treat human faeces so that bacteria are not transmitted to uninfected people	• providing good sewage systems in many developing countries is not economically possible • sewage treatment is often disrupted by natural catastrophes, e.g. earthquakes and floods
make sure that sewage does not contaminate drinking water by having separate sewage and water systems	• many homes in developing countries do not have piped water • rivers may be used both as sewers and sources of drinking water
make sure that people are supplied with clean drinking water which is treated to kill bacteria, e.g. by chlorination	providing water treatment works and piping water to homes is also not economically possible in many places
fast treatment of people infected with cholera by oral rehydration therapy (salts and glucose in sterile water) and, in severe cases, intravenous rehydration and treatment with the antibiotic tetracycline	not always possible for medical staff to reach people in time, especially during natural disasters and other emergencies
vaccinate people to provide active immunity against cholera	the vaccine lasts only for about two years after which time a booster is needed not worthwhile giving cholera vaccine to people as infection is unlikely except in certain circumstances

▼ **Table 10.4** Prevention and control methods for measles

Prevention and control methods for measles	Problems with implementing prevention and control methods
Vaccinate children at 12–15 months and then give a second dose at least 28 days after the first	Vaccination is only effective at preventing outbreaks if 95% of the population are vaccinated. This is not always possible in overcrowded cities and isolated rural areas where there are poor medical services

▼ **Table 10.5** Prevention and control methods for malaria

Prevention and control methods for malaria	Problems with implementing prevention and control methods
stop mosquitoes breeding by destroying breeding areas (e.g. draining marshes) and spraying insecticides	• some areas cannot be drained (e.g. nature reserves) • *Anopheles* mosquitoes breed in very small bodies of water • mosquitoes are resistant to insecticides such as DDT
stop mosquitoes biting at night by using sleeping nets – a method that is most effective when nets are soaked in insecticide every six months	• difficult to provide nets and insecticides to remote and isolated populations • economically/politically difficult in regions where there is civil unrest
• use drugs to stop *Plasmodium* spreading through the human body • use drugs to treat people with malaria so reducing the number of infected people who can pass on the disease to others via mosquitoes	*Plasmodium* has become resistant to many of these drugs, e.g. chloroquine The most effective way to treat malaria is to use the drug artemisinin in combination with other drugs. But *P. falciparum* has become resistant to artemisinins too
vaccines have recently become available	• these vaccines are being trialled or only have limited availability (as of 2017) but may soon become widely available • vaccines may not provide complete protection for everyone • as of 2017 there is no worldwide vaccination programme as there is for polio, measles and many other diseases

▼ **Table 10.6** Prevention and control methods for tuberculosis

Prevention and control methods for TB	Problems with implementing prevention and control methods
quarantine people while they are in the infective stage so that they cannot transmit the disease to others	problems with diagnosing the disease and finding facilities to quarantine people in developing countries
use contact tracing to find people likely to have been infected	only possible where there are health workers capable of doing this
use antibiotics to treat infected people (course of treatment can be 6–12 months); treatment of drug-resistant forms of TB may take longer than 12 months	this is a long course of treatment, many do not finish it – so increasing the risk of drug resistant strains of TB emerging (see page 115)
vaccinate people with BCG vaccine to give them active immunity (see Unit 11 page 125)	the BCG vaccine is not effective across the whole world and its use is now often restricted to use during outbreaks rather than used for providing herd immunity (see Unit 11)
test cattle for TB and destroy any cattle infected with TB; pasteurise milk (TB can be transmitted from cattle to people – see Table 10.2)	TB testing is only possible where there are good veterinary services

▼ **Table 10.7** Prevention and control methods for HIV/AIDS

Prevention and control methods for HIV/AIDS	Problems with implementing control methods
educate the public about 'safer sex' – taking precautions during sex to limit chances of infection	people are often reluctant to change their sexual behaviour
use condoms or femidoms during sexual intercourse	• danger that these may tear during sexual intercourse • not always available for free or at low cost • not always culturally acceptable
identify and treat people who are infected (and are referred to as HIV+)	• only possible where good medical facilities exist • drugs are expensive • no vaccine for HIV
contact tracing to find people likely to be infected	only possible where there are health workers capable of doing this
screen blood donors for HIV refuse donations from 'at risk' groups, such as sex workers treat blood donations and blood products to destroy HIV	routine in many countries, but where these control measures do not happen there is a high risk of transmitting HIV

Worldwide distribution of infectious diseases

Cholera is a disease associated with natural and man-made disasters. Malaria has been eradicated from many parts of the world. TB and HIV/AIDS are found across the whole world although the prevalence is highest in certain regions as shown in Table 10.8.

★ Exam tip

Prevalence is the number of people with a disease at a particular time or within a certain time period, e.g. a year.

▼ **Table 10.8** The worldwide distribution of four infectious diseases (as of 2017)

Disease	Worldwide distribution
cholera	West and East Africa; Afghanistan After natural disasters, war and civil unrest, e.g. Sanna, the capital city of Yemen, in 2017
malaria	Sub-Saharan Africa; parts of South-East Asia
TB	global - highest prevalence in China, India, Sub-Saharan Africa, South-East Asia, The Russian Federation and countries in Central Asia.
HIV/AIDS	global; highest prevalence in Sub-Saharan Africa and South-East Asia

There are many factors that influence the worldwide distribution of these diseases. The World Health Organization (WHO) considers that poverty is a major factor, in both developed and developing countries, that influences the number of cases of these diseases.

Antibiotics

Antibiotics are substances produced by microorganisms (or synthesised chemically) that are used as drugs for the treatment of infectious diseases. Many antibiotics are semi-synthetic because they are modified chemically after production by microorganisms in fermenters, or they are produced entirely by chemical synthesis (although they may have been originally discovered in organisms).

★ Exam tip

You may know the terms bacteriolytic, bactericidal and bacteriostatic for these three ways. You could use technical terms, like these, in your answers if you are sure they are relevant to the questions.

Antibiotics act on bacteria in one of three ways:

- kill bacteria by causing them to burst (lysis)
- kill bacteria without causing them to burst
- stop bacteria reproducing without killing them.

Antibiotics are effective against bacteria and also some fungi. They are not effective against viruses. Antibiotics have their effects by interfering in cell

processes, such as replication and protein synthesis, that occur in bacteria, but *not* in viruses. **Broad spectrum antibiotics** act on a wide range of bacteria. **Narrow spectrum antibiotics** only work on a few.

▼ **Table 10.9** The cellular processes in bacteria that are inhibited by antibiotics

Cellular process in bacteria that is inhibited by antibiotic	Examples of antibiotics
cell wall synthesis	penicillin, cephalosporin, vancomycin
transcription	rifampicin
translation	chloramphenicol, erythromycin, tetracycline, streptomycin
DNA replication	quinolones
cell surface membrane function	polymixin
synthesis of folic acid	sulphonamides, trimethoprin

Antibiotic resistance

Antibiotics are not equally effective against all bacteria. Doctors select the most effective antibiotic for each disease that they treat. Some antibiotics have no effect at all on some bacteria, others may be effective but it is best to find out before prescribing. A sample of bacteria may be taken from a patient and incubated with different antibiotics to find the most effective one to use for treatment. Alternatively, bacteria are tested to see if they have genes coding for proteins that in some way give them resistance to antibiotics.

Bacteria become resistant to antibiotics because of random mutations in DNA. Normally these mutant bacteria do not compete well with the non-mutant ('normal') forms. The normal forms are susceptible to being destroyed by antibiotics.

When someone takes an antibiotic for a bacterial infection, bacteria that are susceptible will die. In most cases if the dose is followed correctly, this will be all the bacteria. However, if the dose is not followed, perhaps because people stop taking the antibiotic when they feel better, then some susceptible bacteria survive and if any mutations occur these might give resistance. The next time there is an infection of this strain of bacteria, the antibiotic may not work as there are some resistant bacteria among those that have infected the body.

In bacteria, a mutant gene has an immediate effect on any bacterium possessing it as most bacteria only have one copy of each gene. These bacterial cells have a selective advantage. Bacteria without this mutant gene will be killed, while those bacteria showing resistance survive and reproduce as they have no competitors. This happens very rapidly: if there was initially only one resistant bacterium, it might produce ten thousand million descendants within 24 hours.

Antibiotic resistance is a serious problem as some bacteria, such as *M. tuberculosis*, show resistance to the drugs, including antibiotics, that are used in treatment. As bacteria become resistant, this makes treating disease very difficult. Antibiotics have been used for over 70 years, so now there are many resistant strains of pathogenic bacteria.

An alarming number of human pathogens have acquired genes to combat all the presently-used antibiotics including vancomycin, which is used as an antibiotic of 'last resort' to treat infections that cannot be cured by other antibiotics. Vancomycin-resistance took 30 years to develop from its introduction. These multidrug-resistant strains are particularly common in hospitals where antibiotic use is heavy, and the patients often have weakened immune systems. One of these is methicillin-resistant *Staphylococcus aureus* or MRSA. This has caused

Remember

Recall from Unit 1 that viruses do not have the cell structures, such as ribosomes and cell wall, that are targeted by antibiotics.

Exam tip

Use the index of this book to find information about transcription, translation and DNA replication. As part of your revision use the topic of *Infectious disease* to revise other areas of the syllabus for Papers 1 and 2.

Exam tip

Antibiotics inhibit processes that do not occur in human cells. Penicillin inhibits the formation of cell walls in bacteria while they are growing. Penicillin cannot act on humans as our cells do not have cell walls.

Exam tip

Anti-viral drugs are not antibiotics. Zidovudine, which is used for treatment of HIV/AIDS, and acyclovir, which is used to treat herpes, are examples of anti-viral drugs.

Exam tip

Resistance genes are often found on plasmids (see Unit 1) which are transferred between bacteria – even between different species.

deaths in hospital patients with suppressed immune systems, for example, people taking immunosuppressive drugs following organ transplants.

To combat antibiotic resistance, it is important that people finish their course of antibiotics to ensure no pathogenic bacteria are left in their bodies. Antibiotics should not be overused; for example, for treating mild complaints and some should be kept 'as a last resort' when all others have failed.

Remember

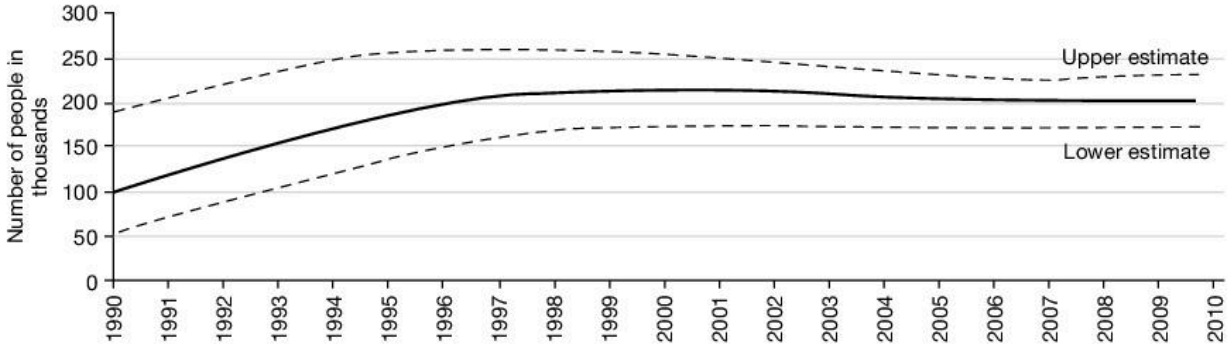
Mutations in bacteria occur by changes to the base sequences of their DNA (see Unit 6 page 70).

Exam tip

There are more ways to reduce the impact of antibiotic resistance than given here. Search the web sites of the WHO and the Centers for Disease Control and Prevention in the USA for more information.

Worked example

Figure 10.1 shows the changes in the estimated number of people infected with HIV in the WHO Caribbean region from 1990 to 2010.



▲ **Figure 10.1**

Exam tip

This question is an example of the type of question that tests your ability to use your knowledge to analyse and interpret unfamiliar information and data.

Use Figure 10.1 to help you answer these questions.

a) (i) Describe the changes in the estimated numbers of people with HIV infection in the Caribbean region between 1990 and 2010.

(ii) Suggest reasons for the pattern you have described.

b) Explain why the upper and lower estimates for HIV infection have been included in the graph.

c) The mortality (death) rate for HIV/AIDS in the Caribbean reached a peak around 2005 and has fallen since. Suggest why the trend for mortality differs from that for prevalence.

Answers

a) (i) The number increases from 100 000 people in 1990 to 210 000 in 1988–89 and decreases to 200 000 in 2005.

(ii) The increase could be due to increased transmission of HIV or it could be due to better diagnosis of people who are HIV positive (HIV+) or people who had been infected with HIV many years before beginning to show symptoms of infection. There is often a period of up to 10 years or more before symptoms of AIDS appear.

b) It is difficult to find out how many people in the population of a region are HIV+. Health services differ in their success at diagnosing people who are HIV+. The numbers collected by different countries may be the result of samples of the population, e.g. people who attend health clinics or hospitals. Many people who are HIV+ do not show any symptoms so they may not be known to the health services.

c) Mortality rates have fallen while the number of people living with HIV has remained constant could be due to better health care. People with HIV can be treated with drugs that prevent the decrease in number of helper T-lymphocytes so that they are not susceptible to opportunistic diseases that are likely to kill them, such as cancers, pneumonia and TB.

Exam tip

The answer to part (c) includes some information from Unit 11, which is required for a complete answer.

Raise your grade

1 (a) Name the organism that causes malaria. [1]

A virus. ✘

If you are asked to *name* the pathogen for any of the diseases given in the syllabus, then you must give the scientific name *in full* and not the type of organism, in this case *Plasmodium falciparum* is a correct answer as well as any of the other species of *Plasmodium* that cause malaria (see Table 10.1).

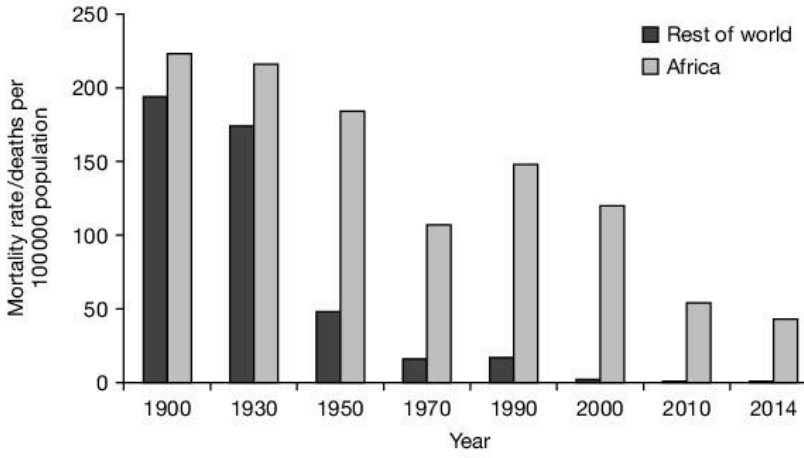
(b) Describe how the organism that causes malaria is transmitted. [3]

Mosquitoes transmit the disease. ✘ They pass it on when they bite people. ✘

Incorrect. The answer should start by stating that the vector is the female *Anopheles* mosquito, which takes a blood meal from someone who is infected with malaria. The pathogen enters the mosquito, reproduces and infective forms of *Plasmodium* enter the salivary glands. When the mosquito feeds again, it injects the infective forms of the pathogen together with anticoagulant into the blood of someone who is not infected. Always refer to transmission between a person *infected* with the pathogen and a person who is *uninfected*.

The 'Roll Back Malaria' partnership is a global programme for restricting the spread of malaria established in 1998. Malaria is particularly important in Africa where trials of vaccines are currently being conducted. The genome of *Plasmodium* has been sequenced and this has made it easier to develop vaccines and drugs for controlling malaria.

The figure shows the changes in mortality from malaria between 1900 and 2014 in Africa and in the rest of the world.



(c) Describe the trends in mortality from malaria in Africa and the rest of the world between 1900 and 2014. [4]

Deaths from malaria have decreased greatly in the rest of the world from about 195 per 100 000 in 1900 to almost nothing in the 2000s. ✔ The mortality rate has always been higher in Africa, decreasing from over 220 to 110 per 100 000 in 1970, ✔ then it increased again to 150 in 1990 ✔ and has decreased again to 40 in 2014. ✔

Correct answer. The candidate has used words like 'increase' and 'decrease' to describe the trends rather than just stating the data, which would not be an acceptable response. It is not necessary to quote the units for every figure when they are the same throughout the question, but they must be made clear at the beginning. A common mistake when reading a graph like this with units stated as 'per 100 000' is to incorrectly give a figure such as 220 000 (Africa in 1930). The death rate in Africa in 1930 was 220 per 100 000 (i.e. 220 people for every 100 000 of the population).

(d) Explain the trends that you have described in **(c)**. [4]

Malaria was controlled in many countries by using insecticides to kill mosquitoes and by removing the places where mosquitoes breed. ✔ These control programmes that targeted the vector were very effective in North America and in Mediterranean countries. ✔ After about 1970, mosquitoes became resistant to insecticides and control programmes were less effective especially in Africa where there were many wars. *Plasmodium* has also become resistant to the drugs used to control it like chloroquine. ✔ There are now drugs available based on artemisinin that are used successfully to treat cases of malaria. These are used in South-East Asia and have reduced the death rates from malaria. ✔

Correct answer. The candidate gives some reasons for the decrease in deaths from malaria in the rest of the world and for the increase in Africa since the middle of the last century and some detailed knowledge such as the name of one of the main drugs used to control malaria. If you are asked to 'explain' you should give reasons. When there are several marks (4 in this question) then you should give at least the same number of different points. Some can be further detail (e.g. chloroquine in this answer) that will often gain marks.

(e) Outline the biological reasons that have made it difficult to develop a vaccine against malaria. [3]

Malaria is an ever-changing disease. *Plasmodium* is eukaryotic ✔ and has a complex genome with many genes. ✔ Some of these genes code for different cell surface antigens. Also, *Plasmodium* has different cell surface antigens at different stages of its life cycle. ✔ This has made it difficult to develop an effective vaccine.

Correct answer. This question is based on a learning outcome in Unit 11 that is relevant here. Do not expect all the parts of each question to be on the learning outcomes in one Unit only.

Exam-style questions

1 What is the name of the pathogen that causes cholera?

A *Vibro cholera*

B *Vibro cholerae*

C *Vibrio cholera*

D *Vibrio cholerae* [1]

2 Which pair of diseases are caused by bacteria?

A cholera and measles

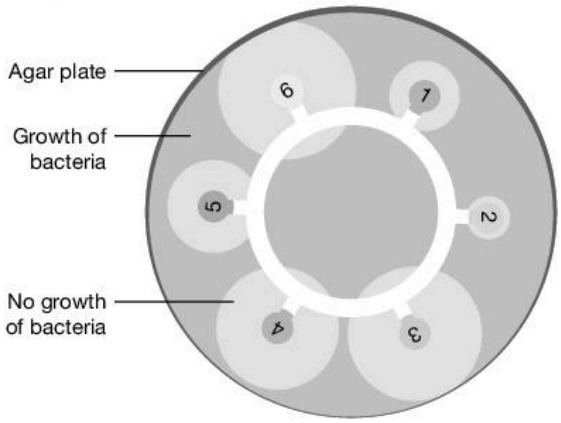
B cholera and tuberculosis

C malaria and measles

D malaria and tuberculosis [1]

3 A patient in hospital had a bacterial infection that was proving difficult to treat.

A medical technician collected a sample of bacteria from the patient. The bacteria were spread over the surface of an agar plate. Six paper discs, each soaked in a solution of a different antibiotic (1 to 6), were placed on the agar plate. The figure shows the growth of the bacteria on the plate after 24 hours.



Which combination of antibiotics is most likely to be effective in treating the bacterial infection?

A 1, 4 and 5

B 1, 2 and 6

C 2, 3 and 5

D 3, 4 and 6 [1]

extra questions available online

4 What is most likely to increase the risk of antibiotic resistance spreading in a population of humans?

A an increase in the incidence of viral diseases

B an increase in the recruitment of doctors

C overuse of antibiotics by the population

D successful vaccination campaigns [1]

5 (a) *Mycobacterium tuberculosis* is the causative organism of TB.

Describe how *M. tuberculosis* is transmitted. [2]

(b) State three social factors that have influenced the spread of TB in recent years throughout the world. [3]

Drug resistance is a problem in treating TB, especially the forms of *M. tuberculosis* that are resistant to two or more of the front-line drugs. There are two forms:

- multiple drug resistant strains of tuberculosis (MDR-TB) are resistant to at least the two main drugs used to treat TB – isoniazid and rifampicin
- extensively (or extreme) drug-resistant tuberculosis (XDR-TB) has also emerged as a very serious threat to health, especially of those people who are HIV+. These strains are resistant to first-line drugs and the drugs used to treat MDR-TB.

These resistant strains of TB do not respond to the standard six-month treatment with first-line anti-bacterial drugs and can take two years or more to treat with drugs that are less potent and much more expensive.

In 2012, an estimated 450 000 people developed MDR-TB in the world. It is estimated that about 9.6% of these cases were XDR-TB.

(c) Outline how bacteria can become resistant to antibiotics. [3]

(d) Suggest why TB and many other infectious diseases are treated with combination therapy not a single antibiotic. [2]

(e) Outline the consequences of drug resistance in combatting tuberculosis. [3]

11 Immunity

Key points

- ☐ State that phagocytes (macrophages and neutrophils) have their origin in bone marrow and describe their mode of action.
- ☐ Describe the modes of action of B-lymphocytes and T-lymphocytes.
- ☐ Describe and explain the significance of the increase in white blood cell count in humans with infectious diseases and leukaemias.
- ☐ Explain the meaning of the term immune response, making reference to the terms antigen, self and non-self.
- ☐ Explain the role of memory cells in long-term immunity.
- ☐ Explain, with reference to myasthenia gravis, that the immune system sometimes fails to distinguish between self and non-self.
- ☐ Relate the molecular structure of antibodies to their functions.
- ☐ Distinguish between active and passive immunity and between natural and artificial immunity.
- ☐ Explain how vaccination can control disease and discuss the reasons why vaccination programmes have eradicated smallpox, but not measles, tuberculosis (TB), malaria or cholera.
- ☐ Outline the hybridoma method for the production of monoclonal antibodies and their use in the diagnosis and treatment of disease.

The mode of action of phagocytes

Neutrophils and macrophages are phagocytes that are produced from stem cells in bone marrow. They are released into the blood. Macrophages are long-lived cells that are found in many tissues of the body. Both types of phagocyte engulf pathogens and other foreign particles by endocytosis.

1. **Chemotaxis**. Phagocytes are attracted towards bacteria by chemicals that they release and by proteins in blood plasma that are activated when pathogens enter the body.
2. **Attachment**. Phagocytes have cell surface receptors that bind to antigens on the pathogen.
3. **Engulfing and fusion**. Extensions of the cytoplasm surround the bacteria; membrane fusion engulfs bacteria in a phagocytic vacuole, known as a phagosome.
4. **Killing**. Lysosomes fuse with the vacuole membrane releasing toxic chemicals, such as hydrogen peroxide, free radicals and toxic peptides.
5. **Digestion**. Lysosomes also release the enzyme lysozyme to hydrolyse glycosidic bonds in bacterial cell walls; other hydrolytic enzymes are also released. Products of digestion are absorbed into the cytosol; some may be removed by exocytosis.

Link

Read Unit 4 page 48 to remind yourself about endocytosis. Also see Unit 8 page 93 for details of neutrophils, monocytes and macrophages.

Exam tip

Macrophages add antigenic material from pathogens to proteins on their cell surface – see antigen presentation on page 122.

The number of neutrophils in the blood increases rapidly during an infection as they leave the bone marrow, circulate in the blood and then pass through capillary walls into tissues. They do not live long after engulfing and digesting bacteria. More are produced and released from bone marrow to replace those that die. This explains why the number of white blood cells in the blood increases during infections (see page 123).

Antigens and antibodies

Pathogens are surrounded by large molecules, such as proteins, glycoproteins and polysaccharides. The molecules are recognised as 'foreign' and stimulate the specific defence system to produce **antibodies**. Any foreign substance that has this effect is an **antigen**.

Self and non-self

Macromolecules that belong to the body are known as '**self**' **antigens**. Although they do not stimulate an **immune response** in the body, they can act as antigens in stimulating antibody production if removed and inserted into an animal or into another person. Any macromolecule that is not recognised as 'self' is known as '**non-self**'. The antigens on the surface of pathogens are an example. The entry of 'non-self' antigens stimulates an immune response.

Lymphocytes

There are two types of lymphocyte:

- **B-lymphocytes** (can be called B-cells)
- **T-lymphocytes** (can be called T-cells).

Lymphocytes originate from stem cells in bone marrow.

▼ **Table 11.1** The functions of B-lymphocytes and two types of T-lymphocyte

Lymphocyte	Function
B-lymphocytes	• differentiate into **plasma cells** that secrete antibodies • production of memory B-lymphocytes
helper T-lymphocytes	• respond to non-self antigens presented on the surface of macrophages • secrete cytokines (cell signalling molecules) to stimulate B-cells, cytotoxic T-cells and macrophages • production of memory T-lymphocytes
cytotoxic T-lymphocytes	• respond to non-self antigens on the surface of many body cells • attach to cells infected with intracellular pathogens and kill them • attach to cancer cells and cells in transplanted tissues and kill them • production of memory T-lymphocytes

Key terms

Helper T-lymphocyte: cell that makes and releases **cytokines** (cell signalling compounds) to stimulate other classes of lymphocyte.

Cytotoxic T-lymphocyte: cell that attaches to infected cells and kills them.

Key terms

Antibody: protein that is secreted by plasma cells (active B-cells) in response to the presence of an antigen.

Antigen: any macromolecule, e.g. polysaccharide or protein, that stimulates the production of antibodies.

Immune response: series of events that occur in the immune system in response to the presence of a non-self antigen in the body.

Key terms

B-lymphocyte: cell that has the potential to make and release antibody molecules.

T-lymphocyte: cell that has the potential either to release cell signalling molecules (cytokines) to coordinate immune responses or to kill infected cells. T-lymphocytes *do not* release antibodies.

★ Exam tip

Take care how you use the terms pathogen, antigen and antibody. Pathogens are organisms; antigens are macromolecules of various types and antibodies are proteins produced by plasma cells (activated B-cells) in response to antigens. Also, it is easy to write the word *antibiotic* instead of antibody. Always reread your answers to check for errors like this.

Key term

Plasma cell: short-lived **activated B-lymphocyte** that makes and releases antibodies into blood and lymph.

Immune responses

Immune responses involve the activation of clones of lymphocytes by the presentation of antigens. When a pathogen enters the body for the first time there are very few lymphocytes with the specific membrane receptors that have a complementary shape to a particular antigen on the surface of the pathogen.

Each small group of lymphocytes is a clone as they all express the same membrane receptor. The activation of clones of lymphocytes that have membrane receptors complementary to a particular antigen is **clonal selection**. To be effective, many more cells need to be produced. In **clonal expansion**, the activated lymphocytes divide by mitosis to form many more identical cells, all with the same specificity.

One type of immune response involves the production of antibodies. After clonal expansion, B-cells differentiate into plasma cells,which quickly produce antibodies. After a short while they die. Other cells in each clone do not become active, but remain in the body, often for many years. These cells are **memory B-lymphocytes (memory B-cells)** and they represent a long-term increase in the number of cells in the original clone.

When antibodies combine with antigens they form antigen–antibody complexes, which phagocytes can then engulf and destroy. Antibodies are very effective against pathogens while they are in the blood, lymph and between cells within tissues. They are of limited use in protecting against intracellular pathogens as, being protein, they cannot cross cell membranes.

The role of T-lymphocytes in immune responses

Pathogens are ingested by phagocytes and are partly digested. Their antigens are presented on the surface of macrophages and other antigen presenting cells (APCs). Helper T-cells with membrane receptor proteins complementary to the antigens, bind to the cell surface of these APCs. This interaction stimulates the helper T-cells to secrete cytokines that activate specific B-cells to divide and differentiate into plasma cells. They also stimulate macrophages to be more effective at killing the pathogens within them. The clone of helper T-cells also divides by mitosis, so that there are more of these cells to increase their stimulatory effect on macrophages and many also remain in the body as memory T-helper cells.

Another type of immune response involves cytotoxic T-cells, which are the most important defence against intracellular pathogens. B-cells and antibodies are not involved in this type of immune response. These pathogens give away their presence within cells as some of their antigens appear in the cell membranes of host cells. For example, during processing in the Golgi body, viral proteins attach to membrane proteins and are exposed at the cell surface when Golgi vesicles fuse with the cell surface membrane. This is another example of **antigen presentation**. The antigen is detected by patrolling cytotoxic T-cells with the specific membrane receptor protein.

Once activated, cytotoxic T-cells attach to the surface of the infected cells, they release perforins, which are proteins that make holes in the cell surface membranes of infected cells. Toxins, such as hydrogen peroxide, pass from the cytotoxic cells into the infected cells and kill them. This is the only way to remove intracellular pathogens, such as viruses and some bacteria. It also prevents reproduction of the pathogen and the spread of pathogens to other cells in the body.

Key terms

Clonal selection: activation of lymphocytes that have membrane receptors complementary to the antigen displayed by pathogens and antigen presenting cells.

Clonal expansion: increase in number of selected lymphocytes (B-cells and T-cells) by **mitosis** to produce activated cells and memory cells.

Key terms

Memory cell: a B-lymphocyte cell or a T-lymphocyte (B- or T-cell) that remains in circulation after an immune response to a specific antigen.

Antigen presentation: the display of antigens on the surface of antigen presenting cells, e.g. macrophages act as APCs to activate to T-lymphocytes.

★ Exam tip

Search online for a transmission electron micrograph of a plasma cell. Make a labelled diagram of the cell. Annotate your diagram to show how it is adapted for protein synthesis. Your annotations will help your revision of many aspects of Units 1, 2 and 11 for Papers 1 and 2.

Immunological memory

Infection by the measles virus tends to give long-term immunity to reinfection by the same pathogen. This is because in the first infection, during the the clonal expansion stage, memory B-cells and memory T-cells are produced in addition to plasma cells and activated helper T-cells and activated cytotoxic T-cells. Memory cells do not differentiate, but continue to circulate in the blood and lymph often for years, possibly a lifetime. If these cells contact the same antigens, then there is another immune response in which these memory cells divide and differentiate. Memory B-cells form plasma cells; memory T-helper cells form active T-helper cells and memory cytotoxic T-cells form active cytotoxic T-cells.

Memory cells belong to a much larger clone of cells than existed before the first infection so they can respond much faster during second and subsequent infections (see Figure 11.3).

★ Exam tip

Memory cells are produced during the first (primary) response to a specific antigen (see Figure 11.3).

Diseases of the defence system

The defence system, in common with other systems of the body, is at risk of developing diseases, including: cancers of the lymphocytes, HIV infection of helper T-lymphocytes, and autoimmune diseases.

Cancers of white blood cells

Stem cells in the bone marrow are responsible for producing white blood cells, such as neutrophils and lymphocytes. These cells can become cancerous. When this happens they divide uncontrollably to produce many cells that do not fully differentiate but leave the bone marrow and enter the circulation. Cancers of stem cells that produce white blood cells in the bone marrow, are **leukaemias**.

- Myeloid leukaemia is a cancer of the stem cells that produce neutrophils.
- Lymphoblastic leukaemias are cancers of the stem cells that produce lymphocytes.

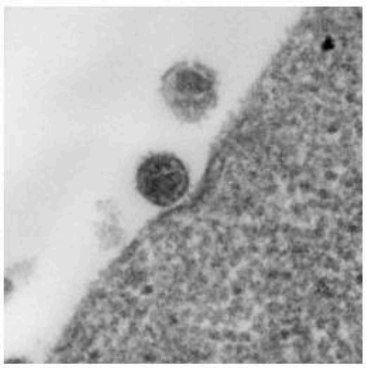
Samples of blood taken from people with leukaemia often have large numbers of undifferentiated white blood cells. They are therefore more susceptible to infections as these undifferentiated cells do not function as normal lymphocytes.

Key term

Leukaemia: a cancer of **stem cells** in **bone marrow** that produce white blood cells.

HIV infection of helper T-lymphocytes

HIV infects a few cell types, including helper T-cells. The glycoprotein (known as gp120) on the surface of HIV binds to a receptor protein known as CD4 to gain entry into the cell by endocytosis (Figure 11.1). HIV infects the helper T-cells. In most cases, HIV is dormant within cells but at some point becomes active and viral replication occurs. As the host cells produce viruses, they often burst open. This decreases the number of helper T-cells. Doctors treating HIV-positive patients monitor the progress of the infection by monitoring the T-cell count in the blood.



▲ **Figure 11.1** HIV particles binding to CD4 proteins on the cell surface (×70 000)

Autoimmune diseases

The immune system sometimes does not function properly. Occasionally T-cells and B-cells do not distinguish between self and non-self and start an immune response to the body's own antigens leading to autoimmune diseases. **Myasthenia gravis** (MG) is an example.

Key term

Myasthenia gravis: an autoimmune disease in which an immune response occurs against acetylcholine receptors on muscle fibres.

A motor neurone releases the neurotransmitter acetylcholine into the gap between the neurone and the muscle fibre. The surface of the muscle fibres has acetylcholine receptors that have a specific shape complementary to acetylcholine. When acetylcholine binds to the receptors, the muscle fibres contract. In some people, B-lymphocytes release antibodies against these receptors so that they are blocked. This reduces the stimulation of the muscle leading to MG with symptoms of muscle fatigue and muscle wastage.

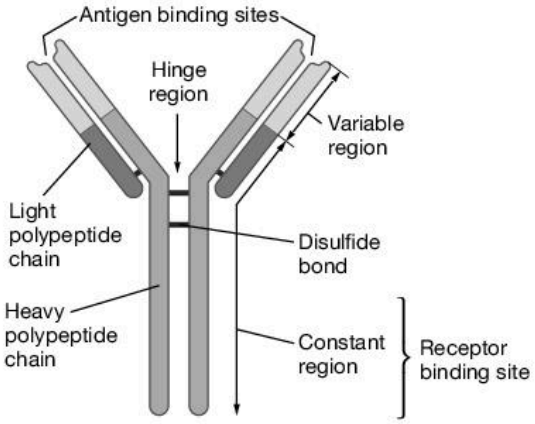
Antibody structure and function

Antibodies are glycoproteins known as **immunoglobulins** (Ig for short). The simplest form of antibody molecule (Ig class G or IgG), and the only one you have to know about, is composed of four polypeptides (Figure 11.2).

Each IgG molecule is composed of two identical long polypeptides (heavy chains) and two identical short polypeptides (light chains).

In order to bind to its specific antigen, each type of antibody molecule has an antigen-binding site that has a specific shape complementary to the antigen. It is possible for different antibodies to have different-shaped binding sites because amino acids can be arranged in different sequences to give different three-dimensional shapes. Because these binding sites vary, these regions are also called **variable regions**.

We need many antibodies with different variable regions to 'fit around' different antigens. The better the 'fit' between antigen and antibody, the more efficient the response to infection.



▲ **Figure 11.2** The structure of an antibody molecule

The polypeptides are joined together by disulfide bonds. The hinge region gives the molecule some flexibility so that the two binding sites can make contact with antigens that may be separated by slightly different distances.

The constant region is the same for all antibodies of the same class. The constant regions of IgG molecules are all identical, whatever the specificities of the variable regions. These constant regions bind to receptors on the surfaces of many cells of the immune system, especially macrophages.

Antibodies have many roles including causing bacteria to clump together so preventing their spread through the body and coating pathogens to facilitate phagocytosis. Antibodies that attach to bacteria help to 'mark' them for destruction by phagocytes. Antitoxins are the antibodies that form complexes with toxins to make them harmless. They are important in the defence against tetanus and diphtheria.

Remember

This is a good opportunity to revise protein structure. To explain how the function of antibodies is related to their structure, you need to know the levels of protein structure. See Unit 2.

Exam tip

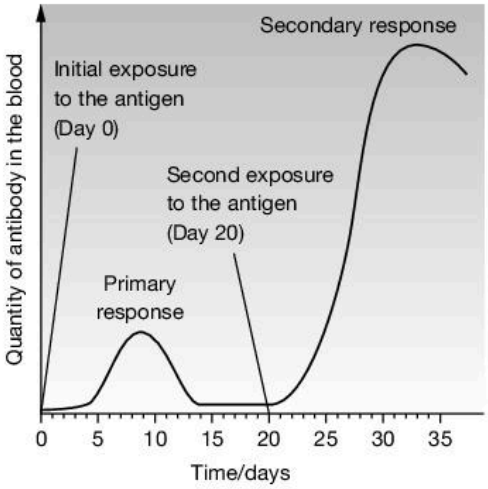
Antigen-binding sites, active sites of enzymes and the binding sites of cell surface receptors all have specific shapes formed by particular sequences of amino acids.

Exam tip

Make a simple labelled diagram of an antibody molecule. Annotate the diagram to describe the function of each labelled part. Use the diagram to describe the four levels of organisation of a protein and the bonds that stabilise the structure.

During the **primary immune response** it takes a while for the specific antibody molecules to appear in the plasma following the presentation of antigen (Figure 11.3). Antigen presentation, clonal selection and clonal expansion have to take place before there are plasma cells able to secrete the appropriate antibody. The concentration increases to a maximum and then decreases as the antibody molecules are removed from circulation.

When a second presentation of the identical antigen occurs, the antibody concentration increases almost immediately in the **secondary immune response**. This happens because there are many more cells (memory cells) of the appropriate B-cell clone to differentiate into plasma cells.



▲ **Figure 11.3** Primary and secondary immune responses

Memory cells are responsible for the secondary response being much faster than the primary response. Notice also in Figure 11.3 that there is a greater response as the concentration of antibodies is much higher. It is unlikely that any infection will become established and that a person has no symptoms during the second and any subsequent infections by the pathogen with that antigen. Antibodies produced by other clones of B-cells that responded to different antigens on the same pathogen will also show the same pattern.

★ **Exam tip**

Place a ruler parallel to the y-axis of Figure 11.3 and move it slowly to the right. Look at the x-axis and the y-axis to identify the two ways in which the secondary response differs from the primary response.

★ **Exam tip**

Make a flow chart to summarise the events that happen during an immune response involving B-cells. Make sure to include four stages: antigen presentation, clonal selection, clonal expansion and the release of antibodies from plasma cells.

Types of immunity

▼ **Table 11.2** The four types of immunity

Type of immunity	Definition	Advantages	Disadvantages
natural active immunity	a person is infected by a pathogen (e.g. *Morbillivirus*), which promotes an immune response	immunity is long-term	immune response takes time; protection not immediate; symptoms develop; disease may be fatal
artificial active immunity	antigens are put into the body in a vaccine (e.g. measles vaccination)	immunity is long-term; no need to suffer from the disease	immune response takes time; protection not immediate
natural passive immunity	antibodies pass from mother to child across placenta and in breast milk especially colostrum (early breast milk)	immediate protection to common diseases which the mother has had or has been vaccinated against	immunity is short-term; antibodies are gradually destroyed in the body; no memory cells are produced
artificial passive immunity	antibodies are injected into a person to give them instant immunity (e.g. antibodies against tetanus toxin collected from blood donations)	immediate protection to specific disease, e.g. tetanus and diphtheria, so person does not suffer from the disease	

Vaccination

A vaccine is the equivalent of the first encounter with an antigen that stimulates an immune response without people being ill. This is equivalent to the primary response (Figure 11.3). A secondary response occurs in a vaccinated person when they encounter the pathogen for the first time, so they are protected against the disease.

Vaccination programmes are an important part of the health protection offered by governments to their citizens. Infants and children are vaccinated against diseases that used to be common in populations and were responsible for much ill health and many deaths.

Mass vaccination schemes give rise to **herd immunity** in which almost all people become immune. People who do not respond to vaccines are protected because the chances of them coming into contact with the disease are small, as most people around them have immunity and will not transmit the disease. In **ring vaccination**, surveillance identifies people who have caught the disease; to prevent it spreading all contacts and people in the neighbourhood are vaccinated.

★ Exam tip

The WHO declared the world free of smallpox in 1980. As of 2017 no other infectious disease has been eradicated from the whole world.

Eradication of smallpox

It was possible to eradicate smallpox because there was only one strain of the pathogen and the virus did not mutate, the virus did not infect animals, it was easy to diagnose the disease, and any person who became infected with the virus developed symptoms of the disease – this meant that there were no carriers to act as a reservoir of viruses that could reinfect people.

The vaccination programme was successful because a live, harmless form of the virus was used in the vaccine ('live vaccine'), which could replicate in the body and produce a higher concentration of antigen to stimulate a strong immune response, so boosters were not necessary; the vaccine was freeze-dried so it was suitable for use in the tropics; and only one vaccine was necessary and so it was cheap to produce.

★ Exam tip

Some vaccines are freeze-dried. This does not mean that they are simply frozen.

Problems with eradication of other infectious diseases

Other diseases have proved more difficult to eradicate than smallpox because some pathogens exist in many strains that keep changing by mutation (e.g. HIV and the virus that causes influenza); the pathogens live in animals, e.g. *Plasmodium* is transmitted by mosquitoes; the pathogens invade the human gut where the immune system does not work very efficiently, e.g. *Vibrio cholerae*; and some vaccines give very short-term protection – the cholera vaccine only works for about a year.

★ Exam tip

Make a large table to summarise information in this Unit about the different cells and molecules involved in the defence system. You can include columns on structure and function. Your table will help with your revision of this topic just before the exams.

It is difficult to develop vaccines against diseases that are caused by eukaryotic organisms, such as the malarial parasite, *Plasmodium*, because they have many genes that code for cell surface antigens. Different strains of *Plasmodium* have different antigens. As *Plasmodium* passes through its different stages in liver and blood cells, it expresses different antigens. It also remains inside liver and red blood cells where antibodies do not have any effect as they unable to enter cells.

Often, in developing countries, it is not possible to vaccinate infants soon enough after birth to protect them from measles infection. After birth, their passive immunity does not last long as the concentration of maternal antibodies decreases. Measles is a very infectious disease and spreads quickly – especially in overcrowded cities.

★ Exam tip

Other reasons for the difficulty with the measles programme are that boosters are required and these are difficult to organise. Children may not have a good diet and their immune response to the vaccine is poor.

Monoclonal antibodies

In the 1970s, scientists in Cambridge, UK, developed a method for producing antibodies from single clones of B-cells. The main problem that they had to overcome was that individual B-cells do not survive if kept in tissue culture. The solution was to fuse them with malignant B-cell tumour cells (known as **myeloma cells**) which survive and divide in culture. This is done by first immunising a small mammal with antigenic material.

After several weeks, B-cells are isolated from the animal's spleen and fused with myeloma cells using chemicals known as fusogens to form **hybridoma cells**, which have genetic material from both cells. These are kept in culture and then each cell is isolated so that the antibodies secreted (if any) can be identified. Cells that produce the required antibody, are grown in culture where they divide and then produce large quantities of this single antibody – a **monoclonal antibody** (often shortened to Mab).

Key terms

Myeloma cell: a cancer cell that divides continuously by mitosis.

Hybridoma cell: a cell produced by fusing together a **plasma cell** (activated B-cell) and a **myeloma cell**.

Monoclonal antibody: antibody of a single specificity secreted by a **clone** of hybridoma cells.

Monoclonal antibodies in diagnosis of disease

There are many different Mabs for diagnosis and for research. Mabs are used in laboratory tests to identify pathogens in samples taken from people suspected of having a disease. These tests are usally ELISA tests, which make use of the high degree of specificity of Mabs to identify specific proteins and other macromolecules. Enzymes, which catalyse a reaction that gives a coloured product, are usually attached to the Mabs. After adding the Mab to a sample and then washing away any Mabs that have not attached to a target, the enzyme substrate is added. If a coloured compound is produced, the test is positive.

A Mab produced against a receptor protein found on the surface of T-cells is used to follow the progression of HIV infections by detecting changes in the number of T-cells in samples of blood. Mabs can also be injected into the body to locate cancer cells or blood clots.

★ Exam tip

Mabs are used in HIV tests to detect the antibodies that people infected with HIV produce.

Monoclonal antibodies in cancer treatment

Mabs can be used to target specific cells in the body. For example, those which express particular cell surface antigens can be located and destroyed by Mabs that also deliver drugs to kill only the targeted cells.

★ Exam tip

Exam questions may be based on examples of Mabs, such as Rituximab for treatment of some leukaemias and Trastuzumab for treatment of some breast cancers. Learn the principles so that you can apply them to any example.

Worked example

a) Explain what is meant by the term monoclonal antibody.

b) Explain why monoclonal antibodies are produced by a process that involves cell fusion.

c) Outline the advantages of using monoclonal antibodies in diagnosis and treatment.

Answers

a) An antibody that is produced by a single clone of hybridoma cells. All the antibodies in any sample of a monoclonal antibody have identical variable regions and so form antigen-antibody complexes with only one type of antigen. They are highly antigen-specific.

b) Antibodies are produced by plasma cells (activated B-lymphocytes). Each clone of plasma cells produces antibody molecules that are all identical. But plasma cells cannot be grown in culture. To make monoclonal antibodies, plasma cells are first fused with myeloma cells that have the ability to grow in culture and keep on dividing.

c) Each type of monoclonal antibody (Mab) is specific to one antigen so can be used to detect the presence of antigens associated with pathogens or with cancer cells. Mabs can also detect very small quantities of antigens so are good for early diagnosis and treatment. In diagnosis and treatment monoclonal antibodies can be injected into the body and will collect at the site of an infection or tumour, so are good for detecting their location in the body and for delivering drugs or radiation treatment.

Raise your grade

1 (a) State the difference between each of the following pairs:

(i) antigen and antibody [2]

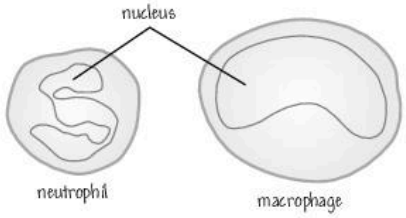
An antigen is any compound that stimulates the production of antibodies.✔ An antibody is a protein molecule produced by plasma cells (activated B-lymphocytes) during an immune response✔.

Correct answer that distinguishes carefully between these two terms.

(ii) macrophage and neutrophil. [2]

Both of these cells are phagocytes. Macrophages are found in tissues and are larger cells with bean-shaped nuclei.✔ Neutrophils are found in the blood and are smaller cells with lobed nuclei✔ (see drawing below).

It is a good idea to include drawings and diagrams in your answers if you can. This helps make it clear what you want to say.



(b) Explain why the secondary response to an antigen is much faster than the primary response. [5]

A primary immune response occurs when an antigen enters the body for the first time. There is a time lag between the entry of the antigen (e.g. when a pathogen invades the body or a vaccine is given) and the appearance of antibodies in the blood plasma.✔ This is because the processes of antigen presentation, clonal selection and clonal expansion have to the place before antibodies are released by plasma cells.✔ During a primary response many memory cells are formed.✔ These are identical to the original cells that responded, but they do not make antibody. When the identical antigen enters the body again, memory B cells respond and become plasma cells✔ which is why the antibody concentration increases much faster in a secondary response because there are so many of them.✔

A very full answer with plenty of detail.

(c) Explain why some people have the disease myasthenia gravis. [4]

Myasthenia gravis is an autoimmune disease✔ in which certain B-lymphocytes detect cell surface receptors on muscle fibres as non-self.✔ The B-cells form plasma cells that produce autoantibodies against the receptors,✔ which respond to the neurotransmitter acetylcholine. When blocked by antibodies, the acetylcholine cannot bind to the receptors✔ and the muscle cells respond less well to nerve impulses.

A good answer that explains the role of the immune system in this autoimmune disease. Acetylcholine is released by motor neurones to stimulate the muscle fibres to contract.

2 (a) Outline the roles of T-lymphocytes in immune responses. [5]

There are two types of T-lymphocyte: helper T-cells and cytotoxic T-cells. Helper T-cells respond to antigens releasing chemicals that stimulate B-cells to divide, become plasma cells and produce antibodies.✔ Cytotoxic T-cells have a different role. They respond to antigens that are on the surfaces of cells. If they recognise the antigen as non-self ✔ then the cells must have a pathogen inside.✔ These T-cells attach to the infected cells and kill them.

A further mark could be gained by describing how cytotoxic T-cells kill infected host cells. The term cytokine should be used in the second sentence to gain a mark.

(b) Describe the hybridoma method of producing monoclonal antibodies. [5]

Inject a solution of antigens into a mouth.✘ After several weeks remove some B-lymphocytes from the spleen✔ of the mouth. These B-lymphocytes will be plasma cells that make antibodies. They are then fused with tumour cells✔ that divide by mitsosis when kept in culture dishes with nutrients. The fused cells are called hybridomas✔ and they have chromosomes from both cells.

The first sentence should read 'Inject a solution of an antigen into a mouse'. Later mitosis has been misspelt, but the this can be ignored and the mark given for identifying the spleen as the source of plasma cells. 'Mitsosis' cannot be confused with anything other than mitosis, so the examiner may award a mark. However, it is easy to misspell mitosis so it looks like meiosis in which case no mark would be awarded. This shows how important it is to reread answers to check for errors.

(c) Outline how children under one year of age may gain passive immunity and artificial active immunity to measles. [2]

- Passive immunity - the child gains antibodies against measles across the placenta before birth and in breast milk.✔
- Active immunity - the child is vaccinated and the measles antigens stimulate an immune response to give long-term immunity (but it needs bossters).✔

There is no need to use bullet points in answering this question, but make sure that **both** parts of the question are answered. Note that there is no point in vaccinating a child against measles while there are still maternal antibodies in the child's body. It would be useless as the maternal antibodies will form complexes with the antigens and there would be no immune response to give active immunity. Boosters (not bossters) are given as part of the vaccination programme for measles.

Exam-style questions

1 Which is the site of phagocyte production in the human body?

A bone marrow

B liver

C lymph nodes

D spleen [1]

2 These cells are part of the body's defence system:

1 B-lymphocytes

2 monocytes

3 T-lymphocytes

Which of these cells produce memory cells?

A 1, 2 and 3

B 1 and 2

C 1 and 3

D 2 and 3 [1]

3 Which of these events happen during clonal expansion during an immune response?

1 cytokinesis

2 DNA replication

3 mitosis

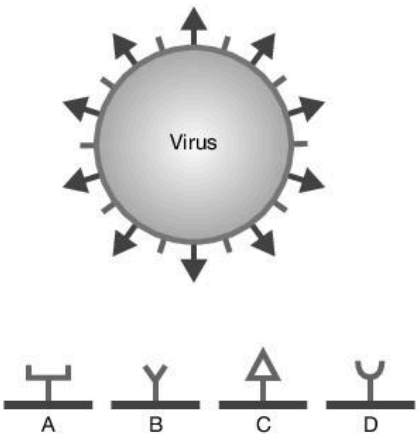
A 1, 2 and 3

B 1 and 2

C 1 and 3

D 2 and 3 [1]

4 The diagram shows the antigens on the surface of a virus and receptors on four clones of B-lymphocytes. Which clone responds when the pathogen enters the body?



5 A student discovered that there are four ways in which a person can become immune.

1 being injected with a vaccine containing isolated antigens from a pathogen

2 being injected with specific antibodies

3 receiving breast milk

4 catching a new strain of a disease

Which of these ways are examples of passive immunity?

A 1, 2, 3 and 4

B 1, 2 and 4

C 2 and 3

D 3 and 4 [1]

6 Soon after a pathogen enters the human body for the first time, the blood contains many different antibody molecules, each produced by a different group of cells.

(a) Distinguish between the terms *pathogen* and *antigen*. [2]

(b) Explain why the response is a *polyclonal response*. [3]

(c) State why these antibodies are present in the blood much sooner following a subsequent infection by the same pathogen. [2]

Monoclonal antibodies are produced for diagnosis and treatment.

(d) Suggest and explain how monoclonal antibodies are used in the treatment of cancer. [5]

7 (a) Explain how it was possible to use vaccination to eradicate smallpox by a worldwide vaccination programme. [5]

(b) A vaccine for measles was introduced in the 1960s. Suggest why it is proving more difficult to eradicate measles compared with smallpox. [3]

12 Energy and respiration

Key points

- ☐ Outline the need for energy in living organisms.
- ☐ Describe the features of ATP that make it suitable as the universal energy currency.
- ☐ Explain that ATP is synthesised in substrate-linked reactions in glycolysis and in the Krebs cycle and that the synthesis of ATP is associated with the electron transport chain (ETC) on the membranes of mitochondria and chloroplasts.
- ☐ Outline the roles of the coenzymes NAD, FAD and coenzyme A in respiration.
- ☐ Explain the relative energy values of carbohydrate, lipid and protein as respiratory substrates and explain why lipids are particularly energy-rich.
- ☐ List the four stages in aerobic respiration (glycolysis, link reaction, Krebs cycle and oxidative phosphorylation) and state where each occurs in eukaryotic cells.
- ☐ Outline glycolysis.
- ☐ Describe the relationship between the structure and function of the mitochondrion.
- ☐ Explain that, when oxygen is available, pyruvate is converted into acetyl (2C) coenzyme A in the link reaction.
- ☐ Outline the Krebs cycle.
- ☐ Explain that reactions in the Krebs cycle involve decarboxylation and dehydrogenation and the reduction of NAD and FAD.
- ☐ Outline the process of oxidative phosphorylation.
- ☐ Explain that during oxidative phosphorylation:
  - energetic electrons release energy as they pass through the electron transport system
  - the released energy is used to transfer protons across the inner mitochondrial membrane
  - protons return to the mitochondrial matrix by facilitated diffusion through ATP synthase providing energy for ATP synthesis.
- ☐ Distinguish between respiration in aerobic and anaerobic conditions in mammalian tissue and in yeast cells, contrasting the relative energy released by each.
- ☐ Explain the production of a small yield of ATP from respiration in anaerobic conditions in yeast and in mammalian muscle tissue, including the concept of oxygen debt.
- ☐ Explain how rice is adapted to grow with its roots submerged in water.
- ☐ Carry out investigations to determine the effect of factors such as temperature and substrate concentration on the rate of respiration of yeast using a redox indicator.
- ☐ Carry out investigations, using simple respirometers, to measure the effect of temperature on the respiration rate of germinating seeds or small invertebrates.
- ☐ Define the term respiratory quotient (RQ) and determine RQs from equations for respiration.
- ☐ Carry out investigations, using simple respirometers, to determine the RQ of germinating seeds or small invertebrates.

ATP and the need for energy

Organisms need a constant supply of energy to stay alive. Energy is needed to do work inside cells and is used for:

- active transport
- muscle contraction
- biosynthesis – the production of biological molecules in anabolic reactions
- raising energy levels of compounds so they take part in reactions
- cell growth and cell division
- maintenance of body temperature in endotherms (animals that use physiological processes to maintain a stable internal body temperature).

Link

Look back to Units 2 and 6 for details of biosynthesis reactions.

Adenosine triphosphate (ATP)

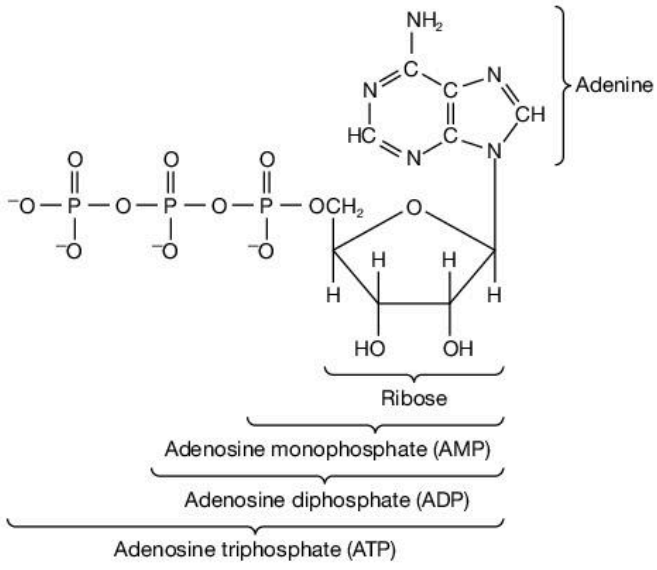
ATP is used by all organisms for energy transfer, hence the term 'energy currency'. ATP is a phosphorylated nucleotide (Figure 12.1). The base adenine and the pentose sugar ribose together form the nucleoside adenosine. With a phosphate added, this becomes a nucleotide. With the addition of two more phosphates, it is a phosphorylated nucleotide.

Remember

See Unit 6 to remind yourself about the structure of nucleotides.

Exam tip

You should be able to make a simple diagram of ATP using shapes to represent adenine, ribose and each phosphate group. See the answer to Question 2 (a)(i) on page 143.



▲ **Figure 12.1** The molecular structure of ATP

ATP is small and water soluble, so it diffuses easily through the cell from sites of production to sites of use. It does not 'leak' out through cell membranes. The bonds between the phosphate groups are unstable and break easily, so ATP can be considered an 'immediate' source of energy. The hydrolysis of an ATP molecule supplies enough energy for an individual step of most anabolic reactions. Many enzymes, and other proteins, in cells recognise the adenine and sugar part of the molecule.

Figure 12.2 shows that the energy required to synthesise ATP comes from the oxidation of energy-rich substances, such as glucose and fatty acids, and from light. The small quantity of ATP in the cell is constantly recycled. When

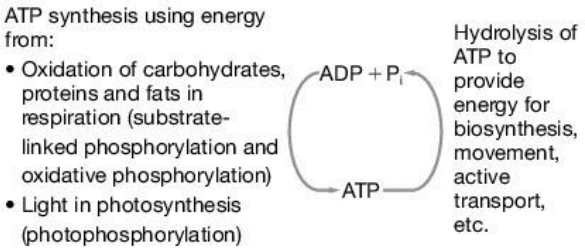
Remember

Remember what you learnt in Unit 3 about active sites. ATP fits into the active sites of many enzymes. Remember also that anabolic reactions are those that make larger molecules, such as protein from amino acids, starch from glucose or nucleic acids from nucleotides.

Key term

Oxidation: the loss of electrons; it always occurs coupled with **reduction**, which is the gain of electrons.

ATP is hydrolysed to ADP and P_i, it releases small manageable 'packets' of energy ($30\,kJ\,mol^{-1}$) in one reaction.



▲ **Figure 12.2** ATP is hydrolysed when it is used to form ADP and phosphate. When reformed, a condensation reaction occurs between ADP and a phosphate ion (P_i). Enzymes catalyse the formation of ATP

There are two ways in which ATP is produced.

Substrate-linked phosphorylation. ADP and a phosphorylated compound are substrates that occupy the active site of certain enzymes. A phosphate group transfers from the compound (shown by XP) to ADP. Production of ATP occurs like this in glycolysis (page 135) and the Krebs cycle (page 137).

Phosphorylation by chemiosmosis. Most ATP in cells is produced using a hydrogen ion (proton) gradient across membranes. This gradient is established by pumping protons from one side of a membrane to the other side. In respiration, energy to do this is provided from reduced coenzymes (page 134) that have been produced by the oxidation of organic compounds (respiratory substrates). In photosynthesis, light is the initial source of energy. This energy is used to produce 'excited electrons' – electrons that have a high energy (page 151).

The protons cannot diffuse back across the phospholipid bilayer of the membrane. Chemiosmosis occurs when the protons move back down an electrochemical gradient by facilitated diffusion through the large membrane protein ATP synthase,which catalyses the phosphorylation of ADP to form ATP.

This equation shows the type of reaction that occurs in substrate-linked phosphorylation:

$$XP + ADP \xrightarrow{\text{enzyme}} X + ATP$$

Key terms

Chemiosmosis: the diffusion of ions (e.g. hydrogen ions) across a membrane down an electrochemical gradient.

ATP synthase: an enzyme found in the membranes of bacteria, mitochondria and chloroplasts that uses the energy from a proton gradient to form ATP from ADP and inorganic phosphate.

Cofactor: any inorganic or organic chemical that is required by an enzyme to catalyse a reaction.

Coenzyme: an organic molecule that acts as a cofactor for an enzyme. It is changed slightly during the reaction.

Coenzymes

Some enzymes do not function unless they are combined with a cofactor. Cofactors may be ions or complex organic substances which may occupy the active site and take part in the reaction or are involved in other ways. Most coenzymes are mobile and travel back and forth between enzymes. Table 12.1 gives the roles of the four coenzymes that you need to know about.

▼ **Table 12.1**

Coenzyme	Role in metabolism
coenzyme A	transfer of acetyl group (2C) in the link reaction of respiration (see page 136) passes 2-carbon compounds from the breakdown of carbohydrate, some amino acids and fatty acids to the reactions of the Krebs cycle
flavin adenine dinucleotide (FAD)	transfer of hydrogen in oxidative phosphorylation in respiration (see page 137)
nicotinamide adenine dinucleotide (NAD)	transfer of hydrogen in glycolysis, link reaction and Krebs cycle in respiration (see pages 135–137)
nicotinamide adenine dinucleotide phosphate (NADP)	transfer of hydrogen between light dependent stage and light independent stage in photosynthesis (see Unit 13 page 151)

Coenzymes NAD, **FAD** and **NADP** are hydrogen carriers which are alternately reduced when they receive a hydrogen atom and oxidised when they pass it on. This allows the transfer of energy via electrons in the hydrogen atoms using different enzymes and is an important part of many metabolic pathways.

The reduced form of NAD and FAD can be written as reduced NAD (or NAD red) and reduced FAD (or FAD red). To include the hydrogen atoms you can write:

$$NAD^+ + 2H \xrightarrow{\text{enzyme}} NADH + H^+$$

oxidised form *reduced form*

$$FAD + H_2 \xrightarrow{\text{enzyme}} FADH_2$$

oxidised form *reduced form*

The changes to NADP may be written in the same ways as those for NAD.

Cellular respiration

Cellular respiration is the transfer of chemical energy from organic molecules so that it is available for cells in a useable form – ATP.

Organic molecules (carbohydrates, proteins and fats) are oxidised so that energy is made available for ATP synthesis (Table 12.2). Carbohydrates (e.g. starch and glycogen) are a short-term store of energy. Fats are long-term stores. Protein may be used as a source of energy if more is present than required for growth, repair and replacement.

▼ **Table 12.2** The energy values of three respiratory substrates

Respiratory substrates	Energy/kJg^{-1}
carbohydrates, e.g. starch, glycogen, glucose, sucrose and lactose	16
lipids, e.g. triglycerides	39
proteins	17

Lipids have much more energy per unit mass than carbohydrates or proteins because they are highly reduced compounds. The three fatty acid chains in each molecule are composed entirely of carbon and hydrogen. When respired, much energy is transferred to molecules of NAD, about twice as much as from the same mass of carbohydrate or protein. Many hydrogens can be transferred for the electron transport chain (ETC) in oxidative phosphorylation.

Table 12.2 shows the energy released when the three groups of substrates are completely oxidised to carbon dioxide and water in **aerobic respiration**.

You only need to know how glucose is respired. There are four stages involved in the aerobic respiration of glucose:

1 glycolysis

2 link reaction

3 Krebs cycle

4 oxidative phosphorylation.

Glycolysis occurs in the cytosol, the other stages occur in mitochondria. If oxygen is not available, respiration can continue but without the use of the stages that occur in mitochondria.

The reactions for aerobic respiration are summarised in this equation:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy}$$

★ Exam tip

The four coenzymes in Table 12.1 are active in plant and animal cells. The first three have roles in respiration. NADP is the only one involved in photosynthesis.

FAD is not mobile but is tightly bound to a Krebs cycle enzyme that is embedded in the inner mitochondrial membrane.

Link

Aerobic is an adjective which literally means 'requiring air', but in biology it refers to requiring oxygen. See Table 12.6 on page 140 for a comparison of respiration in aerobic conditions with respiration in anaerobic conditions.

★ Exam tip

You must remember the overall equation for respiration as you need it to understand the respiratory quotient (RQ) of carbohydrates when they are respired in aerobic conditions (see page 142).

Respiration involves metabolic pathways that need enzymes. In a pathway, the product of one reaction is the substrate of the next. If one reaction in a metabolic pathway is slower than the others, then this is *rate limiting* and slows down the remaining reactions with slow production of the final product.

Glycolysis

Glycolysis prepares glucose for the central metabolic 'hub' of the cell – the Krebs cycle. In animals, glucose can be absorbed from the blood, broken down from glycogen or converted from protein. In plants, glucose can be obtained from the breakdown of sucrose or starch.

In glycolysis, some energy is transferred from glucose directly as ATP and some is 'held' as reduced NAD. The energy is transferred from reduced NAD at a later stage. No carbon dioxide is formed during glycolysis.

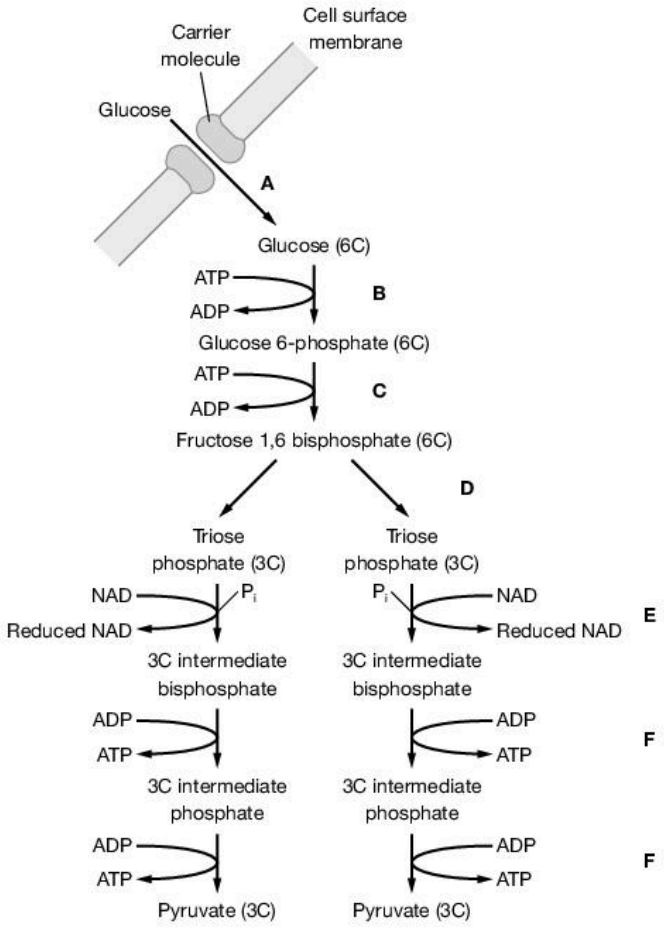
Follow the main stages of the glycolysis pathway in Figure 12.3 and read these descriptions of the following steps carefully.

A Glucose enters the cell by facilitated diffusion through the cell surface membrane.
Glucose is either stored as glycogen or enters glycolysis immediately.

Phosphorylation

B Glucose is phosphorylated to glucose 6-phosphate; this maintains the steep diffusion gradient for glucose to continue entering the cell.

C After another phosphorylation, fructose 1,6-bisphosphate is formed. Phosphorylation produces a more reactive molecule.



▲ **Figure 12.3** The entry of glucose into a cell and the main stages of glycolysis in an animal cell. The number of carbon atoms are shown in brackets

Remember

Remind yourself of the structural formula of glucose from Unit 2 (see Figure 2.2 on page 13). Remember, glucose is a six-carbon sugar.

Exam tip

Copy out the glycolysis pathway onto a large sheet of paper. Make drawings of the simplified structural formulae of glucose, the intermediates and pyruvate using Paper 4 Question 1 on page 252 to help you.

Link

There are many different reactions in glycolysis, each catalysed by a specific enzyme. You should be able to use your knowledge from Unit 3 to explain why different enzymes are needed, not just one. See Unit 3 pages 25–26.

Exam tip

Two molecules of ATP are used per molecule of glucose. See steps **B** and **C** in Figure 12.3.

Exam tip

To help your revision, make a table showing the four stages of respiration, the precise sites in the cell where they occur, the molecule at the start of the stage and the end products.

Lysis (splitting)

D Fructose 1,6-bisphosphate is split into two molecules of triose phosphate (TP). This is the step known as lysis. Note that the reactions after lysis occur *twice* for each molecule of glucose.

Oxidation

E Energy from the two molecules of TP is transferred when it is dehydrogenated. In this reaction the oxidation of TP is linked with the reduction of NAD as hydrogen atoms are transferred from TP to NAD. Energy which would be transferred as heat is conserved by the phosphorylation of TP using phosphate ions that are in the cytosol. Reduced NAD may be used in the production of ATP in oxidative phosphorylation or may be used in other reactions.

Substrate-linked phosphorylation

F ADP and the intermediate triose bisphosphate occupy the active site of an enzyme and one of the phosphate groups is transferred to ADP to form ATP. This is repeated on the active site of another enzyme as the intermediate phosphate and ADP react to give the end product pyruvate and another ATP molecule.

Pyruvate is respired further if there is oxygen available. If not, then it is converted to other substances, for example, ethanol (ethanol fermentation pathway in yeast) and lactate (lactate fermentation pathway in animals).

The structure and functions of mitochondria

The mitochondrion (Figure 12.4) is the organelle in which the rest of aerobic respiration occurs. Glycolysis on its own is not efficient at releasing energy from glucose. Pyruvate is energy-rich and the chemical energy that can be transferred is obtained during the three stages of respiration that occur in mitochondria. The reduced NAD from glycolysis is also a source of energy and it is oxidised and recycled as NAD by mitochondria.

The link reaction

Pyruvate enters mitochondria through carrier proteins in the mitochondrial membranes. In the matrix is a large enzyme complex (pyruvate dehydrogenase) that catalyses reactions that link glycolysis to the Krebs cycle. The components of this complex carry out the following to each molecule of pyruvate:

- dehydrogenation – removal of a hydrogen atom to reduce a molecule of NAD
- decarboxylation – removal of the carboxyl group ($-\overset{O}{\overset{\|}{C}}-OH$) from pyruvate to form a molecule of carbon dioxide
- transfer of the remaining 2-carbon fragment, which is an acetyl (ethanoyl) group, to coenzyme A to form acetyl coenzyme A.

The overall equation for the link reaction is:

pyruvate (3C) + coenzyme A + NAD → acetyl coenzyme A + reduced NAD + carbon dioxide

▼ **Table 12.3** The products of the link reaction

For each pyruvate molecule	For each glucose molecule
1 × carbon dioxide	2 × carbon dioxide
1 × reduced NAD	2 × reduced NAD
1 × acetyl coenzyme A	2 × acetyl coenzyme A

Link

One of the roles of ATP is to raise the energy levels of compounds so that they are more reactive (see page 132).

Exam tip

The products of glucose are 2 molecules of pyruvate, 2 molecules of reduced NAD and 4 molecules of ATP for each molecule of glucose. There is a net gain of 2 ATP.

Link

Some of the reactions of glycolysis are reversible, which is how lactate in animals is converted into glucose (see page 139).

Link

Other reactions/processes occur such as replication of mitochondrial DNA before the mitochondria divide to form two new organelles, and transcription and translation to produce mitochondrial proteins. Chloroplasts also carry out these functions – see Unit 13 page 148.

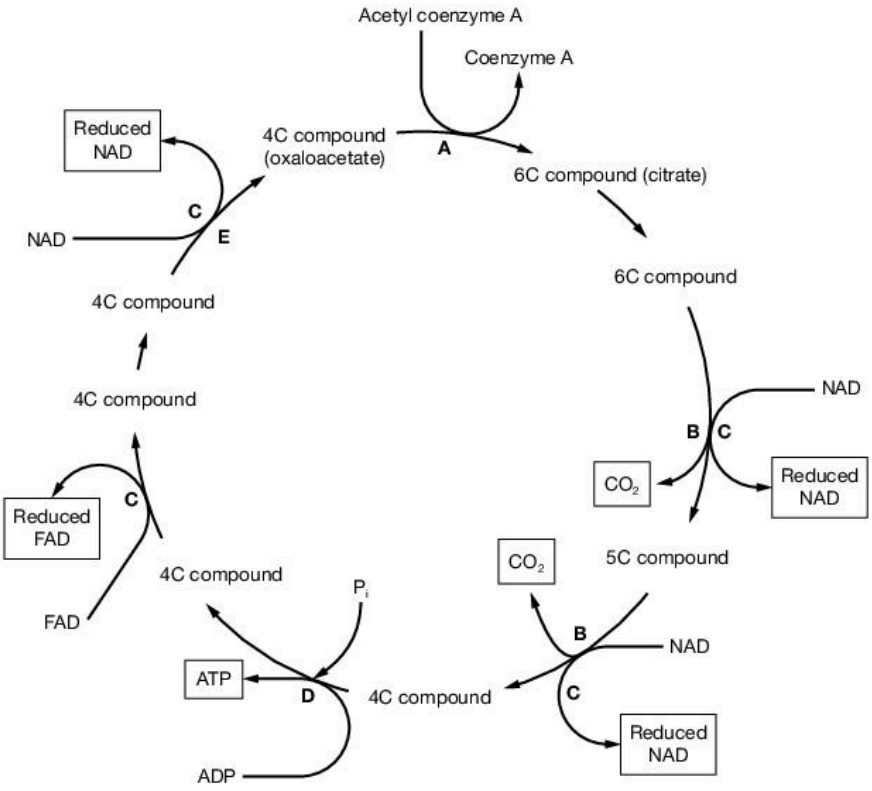
Exam tip

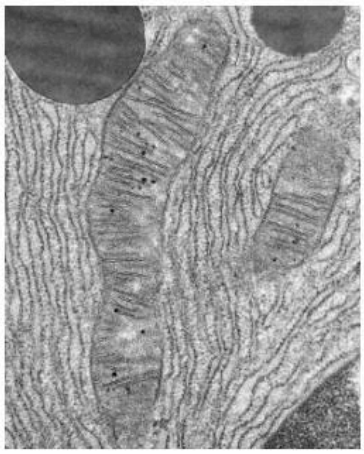
Remember that glycolysis produces two molecules of pyruvate from each molecule of glucose.

Krebs cycle

The two-carbon acetyl group of acetyl coenzyme A reacts in the matrix with a four-carbon compound, oxaloacetate. Oxaloacetate is described as an acceptor molecule. It is recycled by a series of reactions that form the citric acid cycle or Krebs cycle. The main role of the Krebs cycle is the transfer of energy from the intermediate compounds in the cycle to reduce the coenzymes NAD and FAD (Figure 12.5).

Altogether there are nine reactions in the cycle and most of the intermediate compounds are involved in other metabolic pathways.





▲ **Figure 12.4** A transmission electron micrograph showing longitudinal sections of two mitochondria from a pancreatic cell. The mitochondria are surrounded by rough endoplasmic reticulum and secretory vesicles × 25 000

◀ **Figure 12.5** The Krebs cycle. Follow the reactions of the Krebs cycle and see the text for brief descriptions of the reactions, **A** to **E**

A Reaction between acetyl coenzyme A and oxaloacetate (4C); coenzyme A delivers 2C acetyl group into the cycle to form citrate (6C).

B Decarboxylation (× 2); removal of carbon dioxide.

C Dehydrogenation (× 4); removal of hydrogen from intermediate substances, which are oxidised coupled with the reduction of NAD (× 3) and the reduction of FAD (× 1).

D Substrate-linked phosphorylation; ATP synthesis (× 1).

E Regeneration of oxaloacetate (4C compound).

Each reaction of the Krebs cycle occurs twice following the lysis step in glycolysis. This is because two molecules of 3C pyruvate are formed at the end of glycolysis.

▼ **Table 12.4** The processes and products of the Krebs cycle

Process that occurs in the Krebs cycle	Products per one 'turn' of the Krebs cycle	Products per molecule of glucose
decarboxylation	2 × carbon dioxide	4 × carbon dioxide
dehydrogenation	3 × reduced NAD 1 × reduced FAD	6 × reduced NAD 2 × reduced FAD
phosphorylation	1 × ATP	2 × ATP

★ Exam tip

Copy out the link reaction and the Krebs cycle onto a large sheet of paper. Annotate your diagram with information about the processes that occur, the end products and what happens to them.

Oxidative phosphorylation

Glycolysis, the link reaction and Krebs cycle produce reduced NAD and reduced FAD. There is only a small quantity of these coenzymes in a cell, so for the reactions in these three stages to continue, the reduced coenzymes must be oxidised. This oxidation transfers energy to ATP during oxidative phosphorylation – the fourth stage of aerobic respiration.

Exam tip

You can refer to the electron transport chain as the ETC, but write out the name in full first before using the abbreviation.

The oxidation of reduced NAD and FAD provides protons and electrons. The hydrogen from the coenzymes is split into hydrogen ions and electrons and then the electrons pass along the ETC in the inner mitochondrial membrane. The ETC consists of large protein complexes and other molecules that are all components of the membrane. The energy released as electrons pass down the ETC is used to pump protons from the mitochondrial matrix into the intermembrane space to create a proton gradient. When the protons diffuse back to the matrix through ATP synthase, ATP is generated. The energy that drives the whole process derives from the oxidation of glucose during glycolysis.

The ETC is responsible for creating the proton gradient that is put to use in making ATP.

Exam tip

Summarise, with a simple diagram, the exchanges that occur between a mitochondrion and the surrounding cytosol.

Oxygen is the final electron acceptor. Oxygen, protons and electrons together form molecules of water. This reaction is catalysed by cytochrome oxidase, which is part of one of the protein complexes in the inner mitochondrial membrane. The water produced in this reaction is known as metabolic water – a useful source for animals and plants that live in dry habitats.

▼ **Table 12.5** The structure and functions of mitochondria

Structure	Composition	Function
outer mitochondrial membrane	phospholipid bilayer and proteins and some cholesterol	permeable to pyruvate, oxygen, carbon dioxide, ATP, ADP but *not* glucose
inner mitochondrial membrane – folded into cristae (singular: crista) to give a large surface area	phospholipid bilayer with protein complexes of ETC and ATP synthase and some chholesterol	pumping protons into intermembrane space; making ATP; permeable to all of the above, but *not* hydrogen ions or glucose
intermembrane space	lower pH than cytosol and matrix	site of high concentration of protons
matrix	protein-rich region contains DNA loop, ribosomes and many enzyme molecules	link reaction; Krebs cycle
DNA	circular loop of double-stranded DNA (similar to those of prokaryotes); not combined with histone proteins	DNA codes for 13 of the proteins used in the mitochondrion; genes are transcribed as mRNA; rest of mitochondrial proteins are coded by DNA in the nucleus
70S ribosomes	rRNA and proteins	translation – assembly of amino acids to form proteins

Exam tip

Make a large, labelled drawing of a mitochondrion and annotate it with information about the three stages of respiration that occur within it. Use your annotated drawing to help you revise this topic.

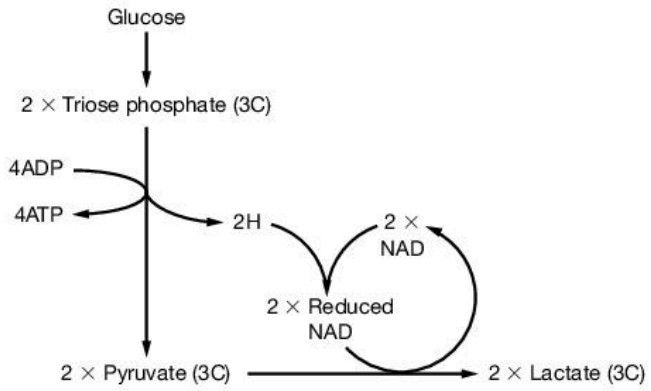
The mitochondrial matrix is the site of the link reaction and Krebs cycle. The products of both stages include the reduced hydrogen carrier, NAD. This is oxidised to NAD and reused in these two stages. The oxidation occurs on the inside of the inner mitochondrial membrane and is catalysed by an enzyme. The cristae provide a large surface for many of these enzymes and other protein complexes of the ETC, so that NAD is recycled quickly.

The coenzyme FAD is part of the ETC. Reduced FAD is oxidised when electrons pass from one complex to another.

Respiration in anaerobic conditions

In the time it takes an elite athlete to run 100 metres (10 s or so), there will not be sufficient oxygen absorbed for aerobic respiration to provide all the ATP needed to provide the energy for the athlete's muscles. Glycolysis responds quickly to the demand for extra energy. The rate of the reactions in glycolysis increases to increase the rate of production of ATP. But oxygen cannot be supplied to mitochondria fast enough for aerobic respiration. As there is insufficient oxygen, the final reaction of oxidative phosphorylation does not take place. There is no other way for the reduced hydrogen carriers to be oxidised, so Krebs cycle and the link reaction stop as they lack NAD and FAD.

This also means that the mitochondria are unable to recycle the reduced NAD from glycolysis. Fortunately, the enzyme **lactate dehydrogenase** catalyses the reduction of pyruvate to form lactate (Figure 12.6). Pyruvate acts as a temporary hydrogen acceptor to form lactate, which starts to accumulate in the muscle cells and then diffuses into the blood.



▲ **Figure 12.6** Lactate fermentation. The pathway that produces lactate in mammalian muscle tissue when there is no oxygen available

The overall equation for lactate fermentation in muscle tissue is:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH(OH)COOH$$

with a net gain of 2 ATP. Note that no carbon dioxide is produced.

Lactate fermentation in muscle tissue is useful because it produces ATP very quickly to provide energy to support exercise. However, the build-up of lactate in muscle tissue lowers the pH and reduces the efficiency of enzymes, making us feel tired. During a sprint, there is an increase in demand for energy, but not an increase in oxygen to supply aerobic respiration at the rate required to supply ATP. All the energy for sprinting comes from the respiration of glucose provided by glycogen stores in muscle, not from fat.

Oxygen debt

At the end of a race an athlete will have built up an oxygen debt. Therefore people continue to breathe deeply after taking a short burst of strenuous exercise.

In the liver, lactate dehydrogenase catalyses the reaction in which lactate is dehydrogenated to form pyruvate. Some of the pyruvate is converted to glucose by the reverse of the reactions of glycolysis. Energy for this is provided by the oxidation of pyruvate in mitochondria. If a runner starts a long-distance event at a fast speed, then he or she will have to slow down to a speed that is supported by aerobic respiration. Respiration has to be aerobic for three reasons. There is not enough glycogen stored in the body to provide

Exam tip

To help your revision, make a table showing the products of each of the four stages of aerobic respiration per molecule of glucose.

Exam tip

A common question on this topic is: 'Describe the fate of pyruvate when there is no oxygen in muscle tissue'. The word 'fate' may seem odd. It means what happens to pyruvate under these conditions.

Link

Creatine phosphate is a readily available store of phosphate that can be transferred to ADP when there is a high demand for energy in muscle tissue. It is not a very large store.

Key term

Oxygen debt: the volume of oxygen that is absorbed to support the metabolism of all the lactate that is produced by muscle tissue during exercise. This is breathed in after exercise stops.

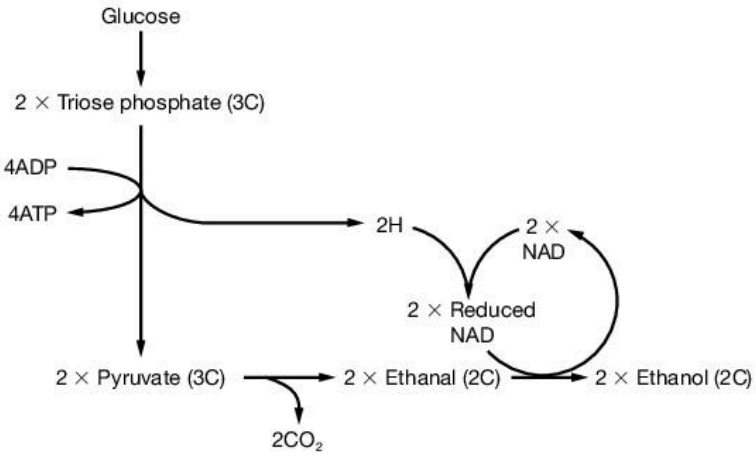
Link

Most intracellular reactions are reversible; the enzymes catalyse forward and back reactions. The direction that a reaction takes is determined by the concentration of substrate(s) and product(s).

the glucose for such a long time. Fat can only be respired aerobically. No-one could tolerate the high concentrations of lactate that would be produced if he or she ran at very high speeds.

Ethanol fermentation

Yeast is a unicellular fungus. Like many microorganisms it can respire with and without oxygen. If no oxygen is present, pyruvate is decarboxylated to form ethanal. Ethanal is then reduced to become ethanol with the oxidation of reduced NAD (Figure 12.7).



▲ **Figure 12.7** Ethanol fermentation. The pathway that produces ethanol in yeast and in plant tissues when there is no oxygen available

Remember

Two molecules of ATP are used in the early stages of glycolysis. See Figure 12.3, page 135.

These reactions that convert pyruvate to ethanol are not reversible. Yeast cannot respire ethanol.

The overall equation for ethanol fermentation in yeast is:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

with a net gain of 2 ATP.

Some varieties of rice, *Oryza sativa*, are adapted to grow in fields that are submerged in water for part of the growing season. Rice has a variety of adaptations for survival in these conditions including:

- hollow stems – oxygen from photosynthesis and from the air can diffuse down towards the roots
- aerenchyma tissue – with large interconnecting air spaces to aid oxygen diffusion throughout the roots to support aerobic respiration.

▼ **Table 12.6** Respiration in aerobic and anaerobic conditions

Feature	Type of respiration		
	respiration in aerobic conditions	respiration in anaerobic conditions	
		ethanol fermentation in yeast and plants (e.g. rice)	lactate fermentation in mammals
decarboxylation	yes	yes	no
oxidation of reduced NAD	yes – in mitochondria using ETC	yes – in cytosol by using ethanal as hydrogen acceptor	yes – in cytosol using pyruvate as hydrogen acceptor
products per molecule of glucose	6 × H_2O 6 × CO_2 32 ATP	2 × ethanol 2 × CO_2 4 ATP	2 × lactate 4 ATP
net gain of ATP	30*	2	2

*The actual net gain of ATP in aerobic respiration varies for several reasons. Remember that it is about 30, so that aerobic respiration is about 15 times more efficient in producing ATP compared with ethanol fermentation or lactate fermentation.

Practical skills

Investigating respiration

Using redox dyes to investigate respiration

Methylene blue is a redox dye. It is reduced to a colourless form which can be readily oxidised back to the blue-coloured form:

coloured form of methylene blue $\underset{\text{oxidation}}{\overset{\text{reduction}}{\longleftrightarrow}}$ colourless form of methylene blue

When added to yeast suspensions, methylene blue changes colour as it accepts some of the hydrogen ions and electrons that normally reduce NAD in glycolysis. Methylene blue can be used to investigate the factors that influence respiration in yeast. This procedure uses it to investigate the effect of temperature.

1 Use a syringe to place 10 cm^3 of 10% glucose solution into six test-tubes labelled **A** to **F**.

2 Use a 1 cm^3 syringe to add 1 cm^3 0.005% methylene blue solution to each test-tube.

3 Use a glass rod to stir the contents of each tube so the blue dye is spread evenly.

4 Place the test-tubes into water baths at the following temperatures
10 °C (**A**), 20 °C (**B**), 30 °C (**C**), 40 °C (**D**), 50 °C (**E**) and 60 °C (**F**).

5 Use a syringe to put 1 cm^3 of a 20% yeast suspension into six test-tubes labelled **1** to **6**. Place test-tube **1** in the same water bath with test-tube **A**, **2** with **B** and so on.

6 Start a timer and leave it running throughout the investigation.

7 After five minutes, pour the 1 cm^3 yeast suspension in test-tube **1** into the glucose solution in test-tube **A** and return test-tube **A** to the water bath at 10 °C. Note the time from the timer.

8 Use the glass rod to stir the contents of the test-tube **A**.

9 Repeat steps 7 and 8 with the remaining pairs of test-tubes.

10 Observe the colour of the tubes and record the time when the contents of each test-tube no longer has a blue colour.

11 If there is no change in colour after 20 minutes, record 'no change'.

▼ **Table 12.7** shows some results from a student investigation

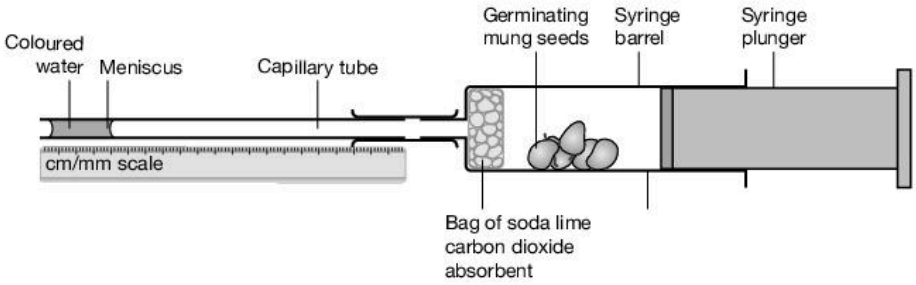
Test-tube	Temperature/°C	Time taken for methylene blue to be decolourised/s	Rate of respiration (1000/t)/s^{-1}
A	10	1080	0.93
B	20	330	3.03
C	30	267	3.75
D	40	187	5.35
E	50	94	10.64
F	60	no change	0.00

The rate of respiration was determined by calculating the reciprocal of the time taken for methylene blue to be decolourised.

The results resemble the effect of temperature on the activity of enzymes. This is because the reactions of respiration are catalysed by enzymes. As the temperature increases, there are more collisions between enzymes and their substrate molecules. At 60 °C all, or almost all, of the enzymes are denatured so there are no or very few enzyme-catalysed reactions.

Respirometers

Simple respirometers (Figure 12.8) are used to measure the rates of respiration of germinating seeds, small animals such as maggots or mealworms and pieces of plant such as leaves. The carbon dioxide absorbent reacts with carbon dioxide to form calcium carbonate. As carbon dioxide is absorbed from the air, the meniscus moves towards the syringe.



▲ **Figure 12.8** A respirometer set up to measure the rates of respiration of germinating mung bean seeds

Calculate the volume of oxygen absorbed using the distance travelled by the meniscus (h) and the radius of the capillary tubing:

$$\text{volume of oxygen absorbed} = \pi r^2 h$$

Respiratory quotient (RQ)

The **respiratory quotient** is the ratio between the volume of carbon dioxide produced in respiration and the volume of oxygen absorbed:

$$\text{RQ} = \frac{\text{volume of carbon dioxide produced}}{\text{volume of oxygen absorbed}}$$

If the RQ is less than or equal to 1, respiration is aerobic. If no oxygen is absorbed, then no aerobic respiration is occurring at all and the RQ is infinity (∞). RQ values higher than 1 indicate a mixture of aerobic respiration and ethanol fermentation in yeast and plants.

The values of RQ of carbohydrate, lipid and protein can be calculated from equations that show their complete oxidation.

This is the equation for aerobic respiration of glucose:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

The ratio between oxygen and carbon dioxide is 1 : 1, so the RQ is 1.

This is the equation for the aerobic respiration of a triglyceride:

$$2C_{57}H_{110}O_6 + 163O_2 \rightarrow 114CO_2 + 110H_2O$$

$$\text{The RQ for tristearin} = \frac{114}{163} = 0.7 \text{ (1 s.f.)}$$

The equation for the aerobic respiration of amino acids (minus the amino group removed by deamination) is:

$$4C_2H_5O_2 + 9O_2 \rightarrow 8CO_2 + 10H_2O$$

$$\text{The RQ for protein} = \frac{8}{9} = 0.9 \text{ (1 s.f.)}$$

RQ values are useful as they tell us whether respiration is aerobic or not, and the type of substrate that is respired (e.g. carbohydrate, lipid or protein) or the likely mix of substrates.

Alternatively, sensors can be used to detect changes in oxygen and/or carbon dioxide concentrations in the air inside a sealed chamber.

Exam tip

Rates of oxygen uptake are often expressed as volume of oxygen per gram of tissue per hour (e.g. $mm^3 g^{-1} h^{-1}$). This involves some careful analysis of the results.

Exam tip

As RQ is a ratio, it does not have a unit.

Link

See Unit 2 page 16 for a description of triglyceride structure.

Exam tip

To measure the rate of carbon dioxide produced use a simple respirometer without carbon dioxide absorbent.

If the meniscus stays in the same place, the volume of carbon dioxide produced is *exactly the same* as the volume of oxygen absorbed.

If the meniscus moves towards the seeds, the volume of carbon dioxide produced is *less* than the volume of oxygen absorbed.

If the meniscus moves away from the seeds, the volume of carbon dioxide produced is *more* than the volume of oxygen absorbed.

Raise your grade

1 During strenuous exercise, such as a sprint, muscles use stores of glycogen.

(a) (i) Describe the changes that occur in the supply of energy within muscle tissue during the first few minutes of strenuous exercise. [3]

The heart beats faster and the rate of breathing increases. This will supply more oxygen to the muscle tissues so they can continue to respire aerobically to supply energy when exercise starts. More glucose is respired so that more ATP is made for muscle contraction.

The question asks about muscle tissue but the candidate's answer is about the supply of oxygen to muscle tissue. When exercise starts, muscle cells use the small quantity of ATP. Not enough oxygen is present for aerobic respiration so lactate fermentation occurs.

(ii) Explain the changes that you have described. [4]

The rate of respiration increases because more energy is needed. The heart and lungs supply more oxygen for oxidative phosphorylation in mitochondria. All the stages of respiration work faster to supply more ATP by substrate-linked phosphorylation and oxidative phosphorylation.

The candidate assumes that the body can respond instantly to the increase in demand for energy by supplying oxygen fast enough to muscle tissues. This does not happen so quickly and the answer needs to explain why lactate fermentation occurs instead of aerobic respiration.

(b) Explain why a person breathes deeply at the end of strenuous exercise. [4]

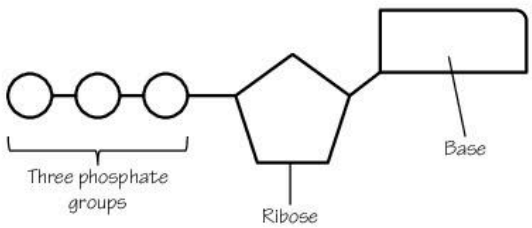
The person has built up an oxygen debt and needs to repay it. ✔ During exercise there was a limited supply of oxygen and lactate fermentation occurred. The lactate cannot accumulate in the muscle cells as it will lower the pH ✔ so it diffuses into blood plasma and is transported to the liver ✔ where it is converted back into pyruvate and either respired completely using oxygen ✔ or is converted to glucose and stored as glycogen.

A good answer. The candidate gives a good explanation of the reason for metabolising lactate quickly at the end of exercise.

2 Most of the ATP produced in an animal cell is formed in mitochondria.

(a) (i) Describe the structure of ATP. [1]

ATP is composed of a nitrogenous base, ribose sugar and three phosphate groups arranged as I have drawn here:



Including a diagram is a good idea, but no mark can be awarded as the base has not been named as **adenine.**

(ii) Explain how ATP is suitable as the universal energy currency in organisms. [3]

ATP is a small, water soluble molecule so can diffuse through cells easily. ✔ ✔ The hydrolysis of the terminal phosphate released enough energy (30 kJ mol^{-1}) for most reactions. ✔ The adenine and ribose 'handle' of ATP fits into many protein molecules (e.g. active site of enzymes). ✔

A good answer that easily scores the marks.

(iii) Explain how the ATP that is **not** produced in the mitochondria of animal cells is formed. [3]

ATP is formed during glycolysis ✔ by the movement of a phosphate group from a 3C compound to ADP. This occurs in the active site of an enzyme and is called substrate-linked phosphorylation. ✔ It occurs four times for every molecule of glucose that is respired. ✔ This makes 4 molecules of ATP, but 2 molecules are used in the first stage of glycolysis so the net gain is 2.

Good answer but the last sentence is unnecessary.

(b) Describe how the structure of a mitochondrion enables aerobic respiration to occur efficiently. You may use a labelled and annotated diagram to help your answer. [4]

Mitochondria have an envelope made of two membranes. The outer membrane has carrier proteins for the transfer of substrates and products of aerobic respiration to pass in and and out. ✔ Pyruvate and ADP pass through these proteins. The inner membrane is folded to form cristae. ✔ This gives a large surface for the protein complexes of the ETC and for the enzyme ATP synthase. ✔ Inside the mitochondria is the matrix that has all the enzymes of the Krebs cycle and link reaction (and other metabolic processes). ✔

A good description.

(c) There are no mitochondria in red blood cells.

Suggest how they respire. [1]

They respire by carrying out glycolysis to make the ATP they need.

This is not enough for the mark. When *only* glycolysis occurs there is no recycling of NAD to keep respiration going. The candidate needs to state that the pyruvate produced in glycolysis is converted to lactate and it is this reaction that recycles NAD for respiration.

Exam-style questions

Structured questions (Paper 4)

1 An investigation was carried out into the production of ATP in mitochondria. A suspension of mitochondria was prepared. To this was added ADP, phosphate ions and plenty of pyruvate and oxygen. The concentrations of these four substances were determined at intervals.

(a) Explain why pyruvate was used as the substrate rather than glucose. [1]

(b) State what you would expect to happen to the concentrations of the four substances added to the suspension and give detailed explanations for your answer. [4]

(c) The experiment was repeated but no phosphate ions were added.

Predict what would happen to the concentration of oxygen in the suspension and explain your answer. [3]

2 NAD and coenzyme A are coenzymes involved in respiration.

The concentration of NAD in muscle is very limited, about 0.8 $\mu mol\ g^{-1}$ of tissue.

(a) Outline how reduced NAD is formed during respiration. [2]

(b) Explain how reduced NAD is recycled in muscle tissue when oxygen is available, and when it is not available. [4]

(c) Describe the role of coenzyme A in respiration. [2]

Free response questions (Paper 4)

3 (a) Glycolysis is the first stage of respiration. State where glycolysis occurs in cells and outline the changes that occur to glucose during glycolysis. [6]

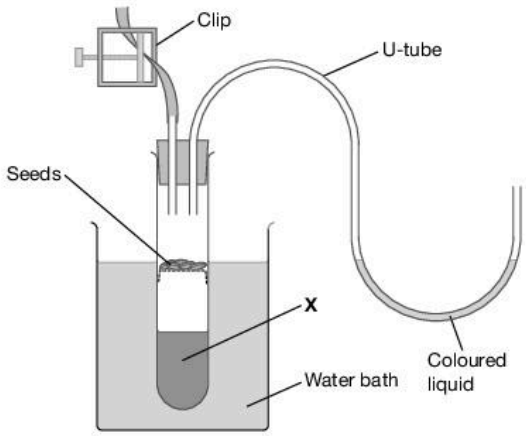
(b) Outline the roles of the Krebs cycle in respiration. [9]

4 (a) Describe the role of mitochondria in respiration. [9]

(b) Explain how rice plants survive when they are submerged in water. [6]

Data analysis and evaluation (Paper 5)

5 The apparatus in the diagram was used to compare the rates of respiration of germinating seeds of pinto beans, *Phaseolus vulgaris*, with the respiration of leaves of the same species.



(a) Name a suitable chemical to use as **X** and explain why it is used. [3]

The apparatus was put into a water bath at 27 °C with the clip open. After 10 minutes the clip was closed and the position of the coloured liquid recorded over time. The results are shown in the table.

Time / minutes	0	5	10	15	20	25	30	35
Position of coloured liquid / mm	0	0	0	31	65	95	130	162

(b) Explain **(i)** why a water bath is used, and **(ii)** why the apparatus is left for 10 minutes before closing the clip. [2]

(c) The diameter of the capillary tube is 0.8 mm. Use the results in the table to calculate the rate of oxygen uptake in mm^3 per hour. Show your working. [3]

(d) Explain how the results would differ if the investigation was repeated at 17 °C. [2]

(e) State how the apparatus would be used to find the rate of respiration of the leaves to make a valid comparison with the beans. Explain your answer. [3]

extra questions available online

13 Photosynthesis

Key points

- ☐ Explain that energy transferred as ATP and reduced NADP from the light dependent stage is used during the light independent stage (Calvin cycle) of photosynthesis to produce complex organic molecules.
- ☐ Describe the relationship between the structure and function of the chloroplast using diagrams and electron micrographs.
- ☐ State the sites of the light dependent and the light independent stages in the chloroplast.
- ☐ Describe the role of chloroplast pigments (chlorophyll a, chlorophyll b, carotene and xanthophyll) in light absorption in the grana of chloroplasts.
- ☐ Interpret absorption and action spectra of chloroplast pigments.
- ☐ Use chromatography to separate and identify chloroplast pigments and carry out an investigation to compare the chloroplast pigments in different plants.
- ☐ Describe the light dependent stage as the photoactivation of chlorophyll resulting in the photolysis of water and the transfer of energy to ATP and reduced NADP.
- ☐ Carry out an investigation to determine the effect of light intensity or light wavelength on the rate of photosynthesis using a redox indicator (e.g. DCPIP) and a suspension of chloroplasts.
- ☐ Outline the three main stages of the Calvin cycle:
  - fixation of carbon dioxide by combination with ribulose bisphosphate (RuBP), a 5C compound, to yield two molecules of glycerate 3-phosphate (GP), a 3C compound
  - the reduction of GP to triose phosphate (TP) involving ATP and reduced NADP
  - the regeneration of RuBP using ATP.
- ☐ Describe, in outline, the conversion of Calvin cycle intermediates to carbohydrates, lipids and amino acids and their uses in the plant cell.
- ☐ Explain the term limiting factor in relation to photosynthesis.
- ☐ Explain the effects of changes in light intensity, carbon dioxide concentration and temperature on the rate of photosynthesis.
- ☐ Explain how an understanding of limiting factors is used to increase crop yields in protected environments, such as glasshouses.
- ☐ Carry out investigations on the effects of light intensity, carbon dioxide concentration and temperature on the rate of photosynthesis using whole plants.
- ☐ Explain how the anatomy and physiology of the leaves of C4 plants, such as maize or sorghum, are adapted for high rates of carbon fixation at high temperatures in terms of:
  - the spatial separation of initial carbon fixation from the light dependent stage
  - the high optimum temperatures of the enzymes involved.

Photosynthesis in outline

The process of photosynthesis may be *summarised* by this equation:

$$n\mathrm{CO_2} + n\mathrm{H_2O} \xrightarrow[\text{chlorophyll enzymes}]{\text{light energy}} (\mathrm{CH_2O})n + n\mathrm{O_2}$$

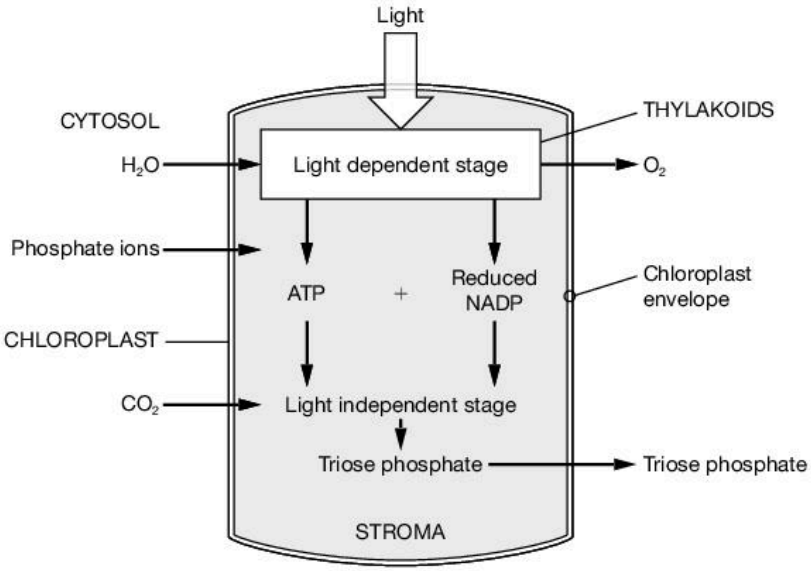
Photosynthesis occurs in two stages in the chloroplasts (Figure 13.1):

- **light dependent stage** in which light energy is absorbed and there is energy transfer with the formation of ATP and reduced NADP
- **light independent stage** in which carbon dioxide is fixed to make carbohydrates.

Key terms

Light dependent stage: reactions that occur in the **thylakoids** of chloroplasts to transfer light energy to chemical energy and so produce ATP and reduced NADP.

Light independent stage: reactions that occur in the **stroma** of chloroplasts to form triose phosphate (TP) using ATP and reduced NADP from the light dependent stage and carbon dioxide.



▲ **Figure 13.1** The sites of the two stages of photosynthesis within the chloroplast and exchanges that occur between them and the rest of the cell

Light energy is absorbed in the light dependent stage. The fixing of carbon dioxide occurs in the light independent stage. This metabolic pathway – the **Calvin cycle** – is cyclic as the acceptor substance is recycled from the products of carbon fixation.

Structure and function of chloroplasts

See Figure 13.2 and Figure 13.3 for the structure of a chloroplast. Table 13.1 describes these structures and outlines their functions.

Exam tip

Compare the structures and functions of chloroplasts with those of mitochondria. Make a list of the similarities and differences.

Exam tip

You will need to learn this equation for $n = 6$. It is important that you know that carbon dioxide and water are the raw materials, simple sugars are the products and oxygen is the by-product of photosynthesis.

Link

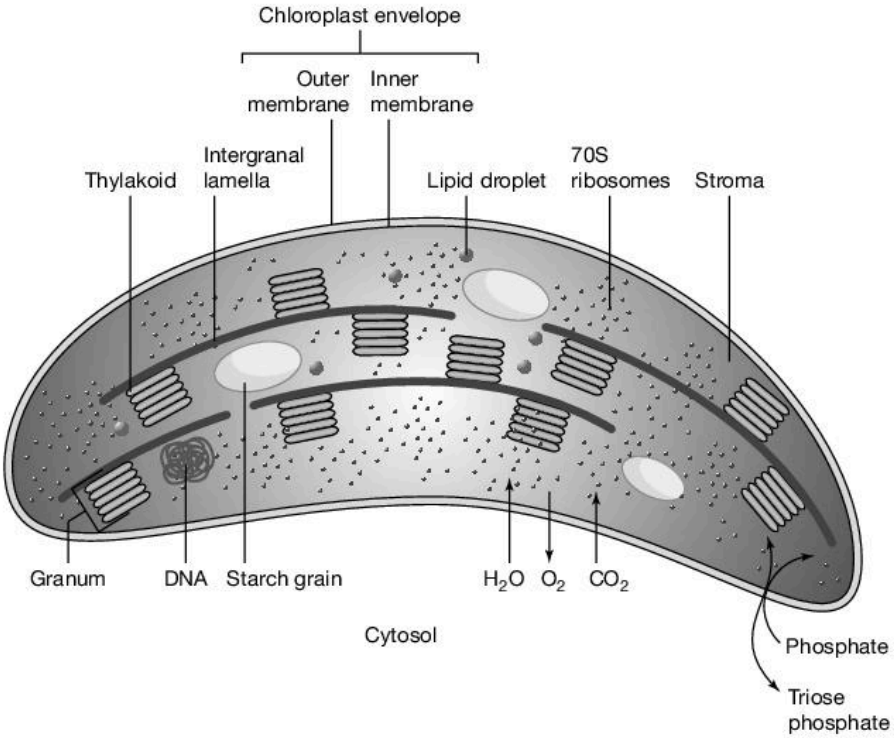
Both stages of photosynthesis occur in chloroplasts. Compare with mitochondria – see Unit 12.

Key term

Thylakoid: flattened membranous sac in a chloroplast that contains chlorophyll and associated molecules needed for the light dependent stage of photosynthesis.

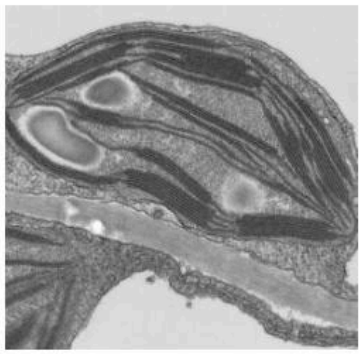
Exam tip

Do *not* use the terms 'light reaction' and 'dark reaction' for the two stages of photosynthesis. Use the light dependent stage and the light independent stage instead.



▲ **Figure 13.3** A drawing of a chloroplast made from a transmission electron micrograph similar to the one in Figure 13.2

Using Figure 13.2, try to identify the structures listed in Table 13.1.



▲ **Figure 13.2** Transmission electron micrograph of a chloroplast

★ **Exam tip**

Make a large, labelled drawing of a chloroplast and annotate it with information about the two stages of photosynthesis. Use your annotated drawing to help your revision of this topic.

▼ **Table 13.1** The structures within a chloroplast, their composition and functions

Structure	Composition	Function
envelope	outer and inner membrane – each composed of a phospholipid bilayer and proteins	protein carriers allow export of triose phosphate and entry of ions, e.g. phosphate, magnesium and nitrate
stroma	colourless, fluid, protein-rich region surrounding the grana; contains DNA, ribosomes and many enzyme molecules	enzymes catalyse reactions to fix carbon dioxide and produce biological molecules, such as lipids, hexoses, starch, amino acids and proteins
granum (plural grana)	stack of membranous sacs called thylakoids	provides a large surface area for light absorption and protein complexes of the light dependent stage
thylakoid	membrane-bound flattened sac, membrane contains photosystems with pigments, electron carriers, proton pumps and ATP synthase	protein complexes pump protons into thylakoid space inside the sac; ATP synthase forms ATP
DNA	circular loops of double-stranded DNA (like those of prokaryotes)	DNA codes for some of the proteins used in the chloroplast; genes are transcribed as mRNA (the rest of chloroplast proteins are coded by nuclear DNA)
70S ribosomes	smaller than ribosomes on endoplasmic reticulum and within the cytosol; same size as those in mitochondria	translation – assembly of amino acids to form proteins

Remember

Thylakoids are like cristae that have split from the inner mitochondrial membrane. The direction in which protons are pumped is the same – from the matrix into the inter membrane space in mitochondria and from the stroma into the thylakoid space in chloroplasts.

Exam tip

Find information about the electromagnetic spectrum to find the wavelengths between the ultraviolet and infrared regions.

Chloroplast pigments

Chloroplasts contain coloured compounds (pigments) that absorb light. Plants absorb light in the visual part of the electromagnetic spectrum between the wavelengths of 400 and 700 nm. The pigments absorb most strongly at either end of this range – in the blue-violet and red regions of the spectrum. When white light is shone onto a suspension of chloroplasts made from a leaf, light in these regions is absorbed and light in the green region is reflected or passes through. Therefore, leaves appear green. Shown on a graph, this pattern of absorption is an **absorption spectrum** (Figure 13.4).

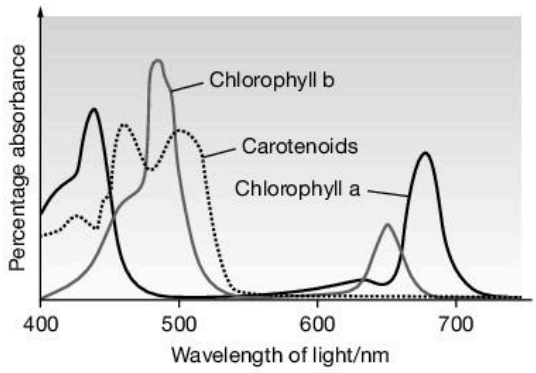
Table 13.2 shows details of the chloroplast pigments. Compare the data for absorption with Figure 13.4.

Key term

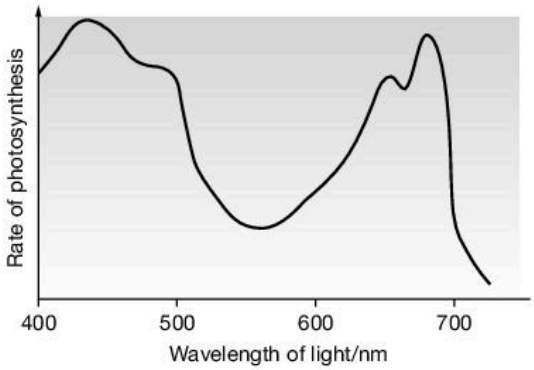
Absorption spectrum: a graph that shows the absorption of different wavelengths of light by a single pigment, a mixture of pigments, a suspension of chloroplasts or by a whole organism.

▼ **Table 13.2** The chloroplast pigments

Pigment	Colour	Peak absorption / nm	Function in photosynthesis
chlorophyll a	yellow-green	430, 662	absorbs red and blue-violet light
chlorophyll b	blue-green	453, 642	absorbs red and blue-violet light
β carotene	orange	450	absorbs blue-violet light
xanthophylls	yellow	450 to 470	may also protect chlorophylls from damage from light and oxygen absorb wavelengths that chlorophylls are poor at absorbing



▲ **Figure 13.4** An absorption spectrum for chlorophylls a and b and carotenoids



▲ **Figure 13.5** An action spectrum

When light of different wavelengths is shone at a suspension of chloroplasts or unicellular algae, the rate of photosynthesis can be determined. The most effective wavelengths are at the blue and red regions and the least effective are in the green region. The pattern shown on a graph is an **action spectrum**. The two spectra are not identical as xanthophylls absorb in the blue and green regions of the spectrum.

Key term

Action spectrum: a graph that shows the activity of a process, such as photosynthesis, at different wavelengths of light.

Practical skills

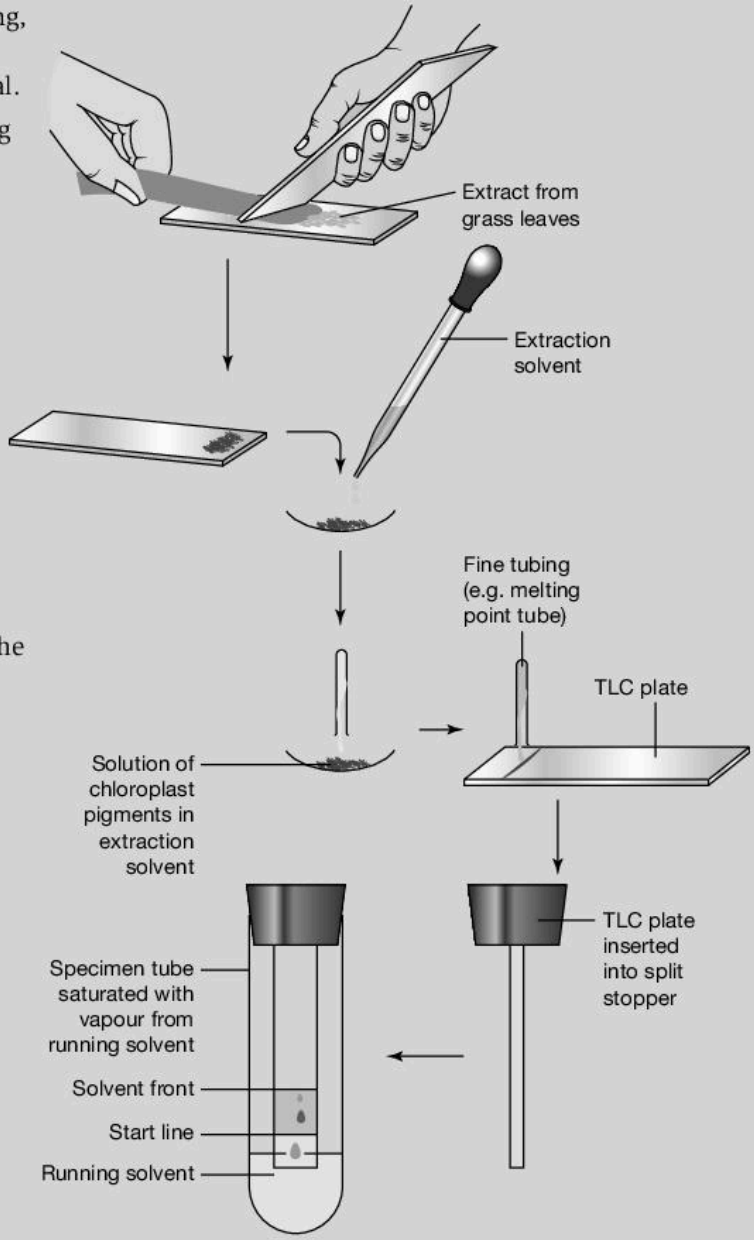
Separating chloroplast pigments by chromatography

Chromatography is a technique for separating, identifying and measuring the quantities of substances extracted from biological material.

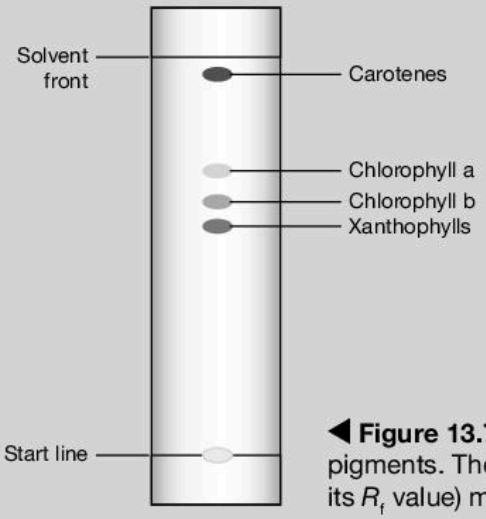
Figure 13.6 shows a procedure for separating chloroplast pigments using thin layer chromatography (TLC). It is a good idea to run pure substances alongside the extract, for example β carotene and lutein (a xanthophyll) to help with identification.

The extraction solvent is propanone (acetone). The running solvent is petroleum ether and propanone in proportions of 2:1.

The solvent front moves up the TLC plate. The plate should be removed when it is about 10 mm from the stopper. The solvent front should be marked immediately with a pencil line. It is a good idea to photograph the chromatogram as the colours fade quickly.



▲ **Figure 13.6**



◀ **Figure 13.7** A chromatogram of chloroplast pigments. The exact position of each pigment (and its R_f value) may vary according to the solvents used

The R_f value (retention factor) is the ratio between the distance travelled by a substance and the distance travelled by the solvent front. It is independent of the length of the chromatogram or the length travelled by the solvent front.

$$R_f = \frac{\text{distance travelled by substance}}{\text{distance travelled by solvent front}}$$

Exam tip

R_f (retention) values do not have units and are expressed as a single number: $R_f = 0$, substance insoluble in the solvent; $R_f = 1$, completely soluble.

The light dependent stage

The pigments in the chloroplast are arranged into **photosystems**. There are two of these photosystems; each one consists of **accessory pigments** and proteins arranged around a **reaction centre** containing a pair of chlorophyll a molecules:

- **photosystem 1** (PSI) – reaction centre (chlorophyll a P700) with a peak absorbance of light at a wavelength of 700 nm
- **photosystem 2** (PSII) – reaction centre (chlorophyll a P680) with peak absorbance of light at a wavelength of 680 nm.

The accessory pigments absorb light of many wavelengths and the energy is transferred to the pair of chlorophyll a molecules in the reaction centres of PSI and PSII.

The energy excites electrons in each chlorophyll a molecule. Figure 13.8 shows the two pathways that electrons can take in the electron transport chain (ETC).

Non-cyclic photophosphorylation

Excited electrons are accepted by one of the electron carriers in the thylakoid membrane. As they pass along the electron transport chain from carrier to carrier, energy is released in small 'packets' and is used by carrier molecules to pump protons from the stroma into the thylakoid space.

The electrons now have a lower energy level when they reach PSI. The absorption of light by this photosystem re-energises the electrons and they are accepted by another electron carrier. From here they travel to NADP, which accepts two electrons and also a proton from the stroma to become reduced NADP. The reduction of NADP is catalysed by the enzyme NADP reductase, which is on the outer surface of the thylakoid membrane.

The loss of an electron from each molecule of P680 in PSII gives it the ability to remove an electron from water. This is aided by the water-splitting enzyme (the oxygen evolving complex, OEC) on the inner side of the thylakoid membrane that catalyses the reaction:

$$2H_2O \rightarrow 4H^+ + 4e^- + O_2$$

Cyclic photophosphorylation

Light energy absorbed by the accessory pigments in PSI is transferred to the reaction centre to excite electrons in P700 in the reaction centre. Electrons travel through the ETC and return to PSI. This flow of electrons provides energy for pumping protons from the stroma to the thylakoid space. Electrons do not reach NADP and there is no photolysis of water.

Protons accumulate in the thylakoid space, which now has a lower pH than the stroma, giving an electrochemical gradient. As in mitochondria, the membrane is impermeable to protons except for channels through the protein ATP synthase. As protons pass down their electrochemical gradient from the thylakoid space to the stroma, the active site of ATP synthase accepts ADP and a phosphate ion. Energy is transferred so a bond forms between the terminal phosphate on ADP and the phosphate ion. This reaction is phosphorylation by chemiosmosis.

The movement of the electrons and the arrangement of the compounds in the ETC are often known as the Z-scheme (Figure 13.8).

Key terms

Accessory pigment: any photosynthetic pigment that absorbs light and passes the energy to reaction centres in PSI and PSII.

Photophosphorylation: the process that takes place in photosynthesis to use light energy to drive the formation of ATP.

Non-cyclic photophosphorylation: involves photolysis of water and production of reduced NADP and ATP. Electrons travel from PSII to PSI and then to NADP.

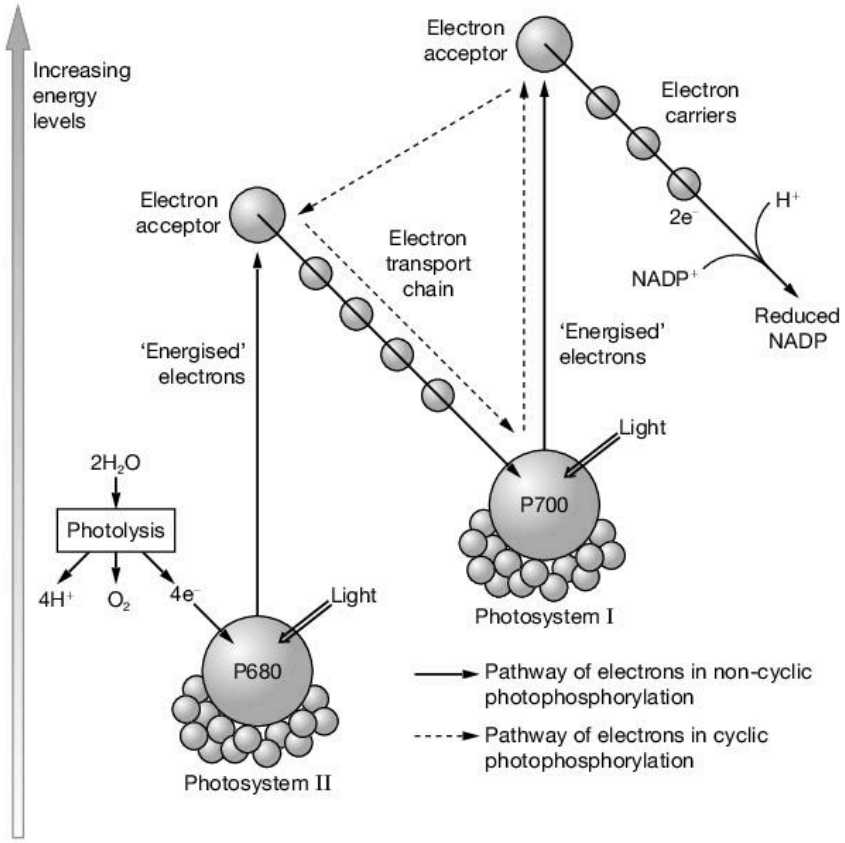
Cyclic photophosphorylation: involves production of ATP without photolysis of water or production of reduced NADP. Electrons travel from PSI but instead of reaching NADP they return to PSI.

Exam tip

The absorption of light and emission of electrons is often called the photoactivation of chlorophyll.

Remember

It is acceptable to write reduced NADP, but you can also use NADPH + H^+.

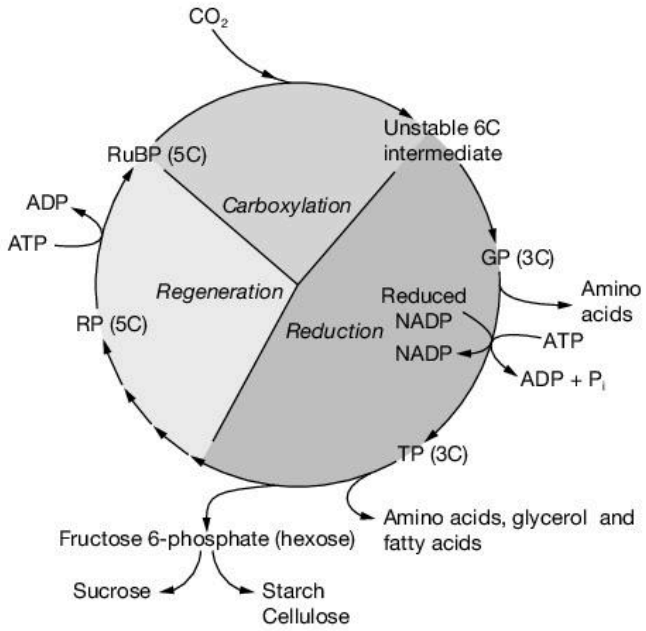


▲ **Figure 13.8** The Z scheme. The energy levels of the photosystems and the electron carriers are plotted with arrows to show electron flow in thylakoid membranes

In the flow of electrons in non-cyclic photophosphorylation, PSII comes before PSI. The reason for this misnumbering is that PSII was discovered first.

The light independent stage

The light independent stage uses ATP and reduced NADP to drive the reactions that fix carbon dioxide and produce sugars that can be used to make all the biological molecules in plants.



▲ **Figure 13.9** The Calvin cycle showing how two intermediates (GP and TP) are converted into amino acids, glycerol, fatty acids and carbohydrates

Link

Plants use the fixed carbon to make the groups of biological molecules that you studied in Units 2 and 6: carbohydrates, lipids, proteins and nucleic acids.

Look at the diagram of the Calvin cycle in Figure 13.9 and find the three main processes that occur:

- **carboxylation** – fixation of carbon dioxide by reaction with the acceptor compound, ribulose bisphosphate (RuBP)
- **reduction** – to form carbohydrates
- **regeneration** – of RuBP using ATP, so completing the cycle.

Molecules of carbon dioxide diffuse into leaves through stomata and then diffuse through the air spaces in the spongy mesophyll. When they reach the cell surface they dissolve in water in the cell wall. They then diffuse through the cell wall, cell membrane and cytosol and through the chloroplast envelope into the stroma.

Carbon dioxide enters the active site of the enzyme ribulose bisphosphate carboxylase/oxygenase (rubisco) together with the 5C compound RuBP. A carboxylation reaction occurs in which a carbon-carbon bond is formed between carbon dioxide and one of the carbons in RuBP. This forms an unstable 6-carbon compound which immediately forms into two 3C compounds known as glycerate 3-phosphate (GP). This substance is the first product of carbon fixation.

Some of the GP molecules are used to make amino acids, but most are reduced and phosphorylated using ATP and reduced NADP from the light dependent stage to make triose phosphate (TP).

TP is a centre of activity of metabolism as it can enter several different metabolic pathways. In the chloroplast, TP can be:

- recycled to RuBP (see Figure 13.9)
- converted into hexose phosphates, which are used to make the polysaccharides amylose and amylopectin (starch for energy storage) and cellulose (for cell walls)
- converted into amino acids and fatty acids
- converted into glycerol and combined with fatty acids to make triglycerides (energy storage) and phospholipids (membranes).

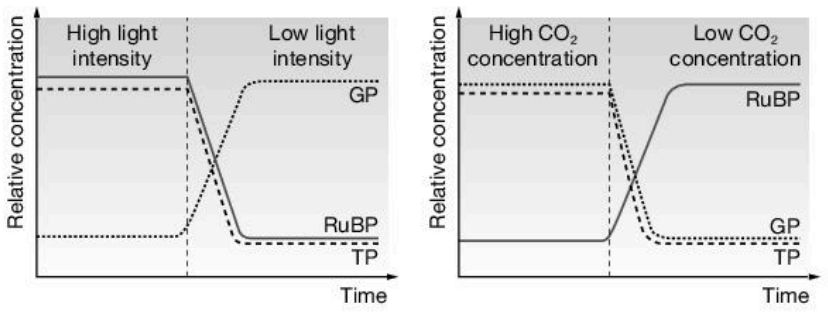
Of every 12 molecules of TP produced, 10 are used to produce 6 molecules of RuBP and 2 may be used to produce hexose or glycerol. Recycling RuBP is important because otherwise it would have to be produced from something else.

Exam tip

Rubisco is the most abundant and most important enzyme on Earth. Remember it is responsible for fixing carbon so plants can make energy-rich compounds that can provide energy, be used for long-term storage and be converted into all the biochemicals, including proteins and nucleic acids.

Worked example

The relative concentrations of three intermediate compounds in the Calvin cycle (GP, TP and RuBP) were determined. The effects of changes in light intensity and carbon dioxide concentration on the relative concentrations of these compounds were investigated. The results are shown in Figure 13.10.



▲ **Figure 13.10**

Explain the changes in the concentrations of GP, TP and RuBP shown in Figure 13.10 **(a)** as the light intensity changes, and **(b)** as the carbon dioxide concentration changes.

Answers

(a) As the light intensity decreases there is less energy to drive the light dependent stage so there is less ATP and reduced NADP produced. This means that less GP is converted into TP, so the concentration of GP increases and the concentration of TP decreases. Also, the reaction catalysed by rubisco (see Figure 13.10, RuBP → GP) continues as carbon dioxide is still available and this increases the concentration of GP. But with decreasing TP, less RuBP can be regenerated so the concentration of GP will reach a constant level and the concentration of RuBP decreases.

(b) When the carbon dioxide concentration decreases there is less carbon dioxide to fix. If there is less carbon dioxide, then RuBP accumulates because it is not being used to fix carbon dioxide. If carbon dioxide is not being fixed, then GP will not be formed, so the concentration decreases, as will that of TP.

Practical skills

Investigating factors that influence the rate of photosynthesis

Rates of photosynthesis can be measured by:

- detecting the activity of the light dependent stage using redox dyes, such as methylene blue and DCPIP
- measuring the production of oxygen in the light dependent stage by collecting the gas (as oxygen is not very soluble in water)
- using sensors to measure the uptake of carbon dioxide in the light independent stage
- measuring the production of carbohydrate by finding increases in dry mass.

Experimental work on photosynthesis often uses suspensions of the single-celled alga, *Chlorella*, or chloroplasts isolated from the leaves of plants such as lettuce and spinach.

Exam tip

Chloroplasts are organelles, so have no cell walls. They are suspended in a sucrose solution with the same water potential as cytoplasm to prevent them bursting.

Rates of photosynthesis using a redox indicator

The transfer of electrons and hydrogen ions to hydrogen carriers (NADP) occurs during the light dependent stage. DCPIP, a blue indicator, acts as a hydrogen acceptor, turning colourless as it is reduced during the light dependent stage.

Exam tip

DCPIP is used instead of NADP so that visual results can be obtained.

Electrons and hydrogen ions from water + DCPIP → reduced DCPIP
(blue) (colourless)

Remember

In Unit 12, methylene blue was used as a redox indicator to investigate the rate of respiration.

In this procedure, chloroplast suspensions with added DCPIP are exposed to different **wavelengths of light**.

1 Put 5 cm^3 of the chloroplast suspension into a test-tube and add 1 cm^3 of water. This tube is a colour standard to help decide when the redox indicator has become colourless.

2 Label five test-tubes A to E. Wrap each test-tube in foil to exclude light.

3 Put 5 cm^3 of the chloroplast suspension into each test-tube and cover with a foil cap or a stopper.

4 Add 1 cm^3 of the medium containing DCPIP to each test-tube.

5 Start a timer and turn on a light source, such as a bench lamp. Make sure there are no other light sources.

6 Remove the foil from test-tube A, wrap a purple filter around the front of the test-tube facing the light source and secure with an elastic band. Put the test-tube in a rack and note the time.

7 Repeat Step 6 with the other test-tubes using blue, green, orange and red filters for these tubes.

8 Record the time when the colour of the suspension in each tube matches the colour standard.

9 The rate of photosynthesis can be determined by calculating the reciprocal of the time taken for the indicator to become colourless.

▼ **Table 13.3**

Colour of filter	Wavelength / nm	Time taken for DCPIP to decolourise / s	Rate of photosynthesis (×1000) / s^{-1}
purple	425	38	26.3
blue	450	83	12.0
green	525	485	2.1
orange	625	46	21.7
red	675	51	19.6

★ Exam tip

Sketch a graph of the results in Table 13.3 and you have an action spectrum. Compare it with the action spectrum in Figure 13.5.

This method can also be used to investigate the effect of **light intensity**. Instead of using coloured filters, use neutral filters that reduce the intensity of light that is transmitted. Or put the lamp at different distances and use a light meter app to record the intensity.

Limiting factors

There are three environmental factors that have most influence on the rate of photosynthesis.

Light provides energy for photosynthesis so as the light intensity increases so does the supply of energy that can be absorbed by chloroplast pigments in the thylakoids (stacked thylakoids are called grana) and used in the light dependent stage.

Carbon dioxide is the raw material for photosynthesis, so as carbon dioxide concentration increases so does the supply of carbon to be fixed in the light independent stage.

Temperature influences the activity of enzymes, so an increase leads to an increase in enzyme activity up to an optimum temperature. Both light dependent and light independent stages involve enzymes, but temperature has a much greater effect on the light independent stage.

★ Exam tip

It is most important that you use **light intensity** and **carbon dioxide concentration** when writing about these limiting factors; 'light' and 'carbon dioxide' unqualified are not correct.

Crop production

Farmers and growers of protected crops (e.g. tomatoes, lettuce, and cucumber) in temperate countries have fully automated glasshouses with sensors and computerised systems that maintain suitable conditions for high rates of photosynthesis. These systems:

- control light intensity using artificial lighting and shading
- control temperature with heaters and ventilation
- enrich the carbon dioxide concentration of the air by burning hydrocarbons (e.g. propane) or using tanks of liquid carbon dioxide
- supply water directly to the roots
- use humidifiers to maintain a humidity appropriate for the crop
- supply mineral nutrients directly to roots at the concentrations appropriate to the growth stage of the crop.

In the tropics, growers use plastic and mesh greenhouses to control the climate. Plastic protects against heavy rain and the mesh provides protection against high light intensities and intense heat so that salad crops are not scorched. Drip irrigation is used to reduce watering costs as water is supplied directly to the plants.

Link

Control systems in glasshouses use negative feedback to maintain constant conditions of light intensity, temperature, carbon dioxide concentration and humidity. See Unit 14 for information on negative feedback.

Growing crops in protected environments has the added advantage of making it relatively easier to control pests and diseases compared with field crops.

Practical skills

Investigating the limiting factors that affect photosynthesis

To determine rates of photosynthesis, collect the gas given off by an aquatic plant such as *Elodea*. Figure 13.11 shows a simple way to do this using the same apparatus as used to make respirometers (see Unit 11). Any gas given off by the plant comes out of solution and collects at the top of the syringe. The increase in volume causes the solution to move down the capillary tube.

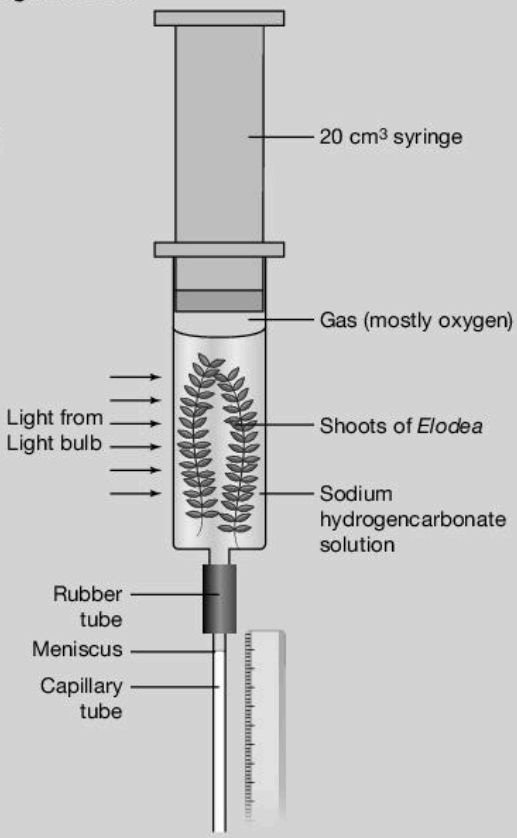
Exam tip

Exam questions on photosynthesis may involve the analysis of data on oxygen production, carbon dioxide uptake or dry mass changes. They will all show the *net* rate of photosynthesis as respiration uses oxygen and carbohydrates and also produces carbon dioxide.

The apparatus in Figure 13.11 can be used to investigate the effects of light intensity and carbon dioxide concentration. As the apparatus cannot be placed inside a water bath, it is not easy to use it to investigate the effect of different temperatures.

Exam tip

You will probably use a different apparatus to investigate the effect of one or more of these limiting factors. This involves putting the aquatic plant in a test-tube kept inside a water bath set at a constant temperature. The gas given off is collected and its volume measured.



▲ **Figure 13.11** Simple apparatus set up to determine the rate of photosynthesis of *Elodea canadensis*

The **light intensity** is changed by using a variable resistor to change the current flowing to the lamp, or by putting the lamp at different distances from the plant. You can measure the light intensity with a light meter app, or, if the distance is varied, by calculating $1/d^2$ where d = the distance between lamp and plant.

Carbon dioxide concentration is changed by making up different dilutions of a stock solution of sodium hydrogencarbonate.

When investigating one variable, e.g. light intensity, the other two variables, carbon dioxide concentration and temperature, must be kept the same. Take these precautions when measuring rates of photosynthesis.

1 Exclude light from other sources. Ideally, cover the windows so the only light source is the lamp.

2 If the same piece of *Elodea* is to be used for some time, then change the sodium hydrogencarbonate solution to maintain a constant carbon dioxide concentration.

3 Keep the temperature constant or record any temperature fluctuations.

4 The plant takes time to adjust to the conditions and to reach a constant rate of photosynthesis for each set of conditions used. Therefore, record the volume of gas produced and only take readings when it has become constant. When it is necessary to use different pieces of *Elodea*, ensure that they each have the same mass.

C3 and C4 plants

The first stable product of carbon fixation by rubisco in the Calvin cycle is the **3C compound** GP (Figure 13.9). Plants that produce this 3C compound in the reaction to fix carbon dioxide are called **C3 plants**. Oxygen competes with carbon dioxide for rubisco's active site. When this happens, rubisco catalyses a different reaction that produces only one molecule of GP and a 2C compound. This reaction reduces the efficiency of photosynthesis.

Many plants use this C3 method, but in hot climates with high light intensities, the production of oxygen is very high and this reduces the efficiency of rubisco considerably. Some tropical species, including important crop plants, use a different enzyme to catalyse carbon dioxide fixation. The carbon acceptor substance (equivalent to RuBP) is phosphoenolpyruvate (PEP) and the enzyme is phosphoenolpyruvate carboxylase (PEPC). The product is oxaloacetate, which is a 4C compound. Plants that use this way to fix carbon dioxide are **C4 plants**.

C4 plants have a Calvin cycle the same as shown in Figure 13.9 and they also use rubisco to catalyse a carboxylation step. The reaction catalysed by PEPC is *in addition* to the reaction catalysed by rubisco. The two stages of photosynthesis occur in different types of cell. Carbon dioxide fixation occurs in chloroplasts in mesophyll cells that exchange gases with the air inside the leaf. The light independent stage (Calvin cycle) is restricted to chloroplasts in bundle sheath cells that have little or no contact with air. Chloroplasts in bundle sheath cells have few thylakoids and no PSII so they do not produce any oxygen by photolysis. This spatial separation ensures that oxygen does not reach rubisco in the bundle sheath cells. Oxaloacetate is converted into malate, which diffuses from mesophyll cells through plasmodesmata into bundle sheath cells where it is broken down to form carbon dioxide to react with RuBP.

Enzymes in C4 plants have higher optimum temperatures than the same enzymes in C3 plants (see Question 2 on page 160). C4 plants are mainly distributed in the tropics as they do not compete well with C3 plants in colder climates.

Key term

C3 plants: plants that have GP (3C) as the product of the reaction that fixes carbon dioxide. These are more common in temperate climates.

Remember

Look again at Figure 13.10 showing the Calvin cycle. GP is the product of the carboxylation of RuBP.

Key term

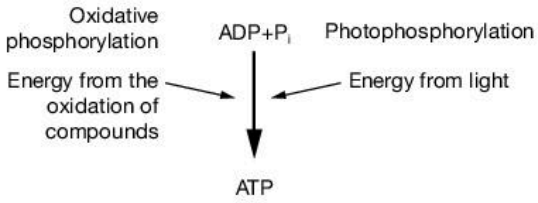
C4 plants: plants that have oxaloacetate (4C) as the product of the reaction that fixes carbon dioxide. These are more common in tropical climates.

Remember

Recent research shows that there is a very high density of plasmodesmata between mesophyll cells and bundle sheath cells – see Units 1 and 7 for details of plasmodesmata.

Raise your grade

1 (a) The figure shows the formation of ATP in a palisade cell.



(i) Explain the term *phosphorylation*. [2]

It is the addition of a phosphate group to a compound, ✔ e.g. ADP gains a phosphate group from an intermediate compound during glycolysis ✔ to become ATP in substrate-linked phosphorylation.

This is a good answer.

(ii) State the precise sites in a plant cell of the two types of phosphorylation. [2]

Oxidative happens in mitochondria; ✘ photophosphorylation in chloroplasts. ✘

This is not precise enough to gain either of the marks. The answers are inner mitochondrial membranes and thylakoid membranes in chloroplasts.

(iii) State three similarities *and* three differences between photophosphorylation and oxidative phosphorylation, *other than* where they occur. [6]

Both processes use the ETC, ✔ ATP synthase is in both organelles to produce ATP ✔ and both have proton pumps to make a high concentration of protons (in the intermembrane space and in the thylakoid space). ✔

These answers are correct but the candidate has forgotten to give any differences.

(b) State five uses of ATP in a plant cell. [5]

DNA replication, ✔ absorbing ions by active transport ✔ and activating amino acids for protein synthesis. ✔

The candidate has only given three uses, not five.

2 (a) The rate of photosynthesis was determined by using discs cut from *Coleus* leaves. The discs were placed in a tube and all the air inside the leaf discs was removed. When placed into a tube of dilute sodium hydrogencarbonate solution the discs sank to the bottom. The discs were kept in the dark and at intervals some were removed and placed in different light intensities. The time taken for five of the discs to float was recorded. The investigation was repeated with freshly cut discs at a higher temperature. All the results are in the table.

Distance of lamp from leaf discs / mm	Time taken for five discs to float at 20 °C / seconds	Time taken for five discs to float at 30 °C / seconds
50	275	125
100	390	210
150	410	360
200	620	600
250	none of the discs rose to the surface	none of the discs rose to the surface

(a) Explain why the leaf discs sink to the bottom of the tube and why they float. [3]

The density of the discs is greater than water. ✔ They have had all the gas removed from the internal air spaces. When the leaves absorb light they use the energy to split water (photolysis) to provide electrons to the ETC. Oxygen is produced as a by-product. ✔ It forms bubbles in the water inside the mesophyll and makes the leaf less dense than water so it floats. ✔

The is candidate has given a very thorough answer

(b) Describe and explain the trend shown by the leaf discs kept at 30 °C. [5]

As the distance of the lamp increases, the time taken for discs to float increases. ✔ At 30 °C the time increases by a factor of 4.8 (50 mm to 200 mm). ✔ The light intensity is highest when it is closest to the leaf discs (50 mm). The discs photosynthesise at the fastest rate as light provides energy for the light dependent stage which is where photolysis occurs. As the distance increases, the light intensity decreases ✔ and so the rate of photolysis decreases ✔ and it takes longer to make enough oxygen to reduce the density of the leaves so that they float. ✔

Before answering, annotate the table to look for the trend, e.g. you could put an arrow down the table indicating decreasing light intensity. Notice that the table has 'distance' as the independent variable. If you calculate light intensity as $1/d^2$ then the shortest distance (50 mm) has the highest light intensity. This is a good answer that includes some manipulation of the data (×4.8) and explains the effect of *decreasing* light intensity with increasing distance.

(c) The leaf discs left in the dark remained on the bottom of the tube. Explain why they did not float. [3]

The discs do not photosynthesise in the dark ✔ so no oxygen is made in photolysis. ✔ The cells still carry out respiration so they will use any dissolved oxygen ✔ and make carbon dioxide, which is much more soluble in water than oxygen so stays in solution.

A detailed explanation. Full marks.

(d) Explain why the leaf discs kept at 20 °C took longer to float than those at 30 °C. [3]

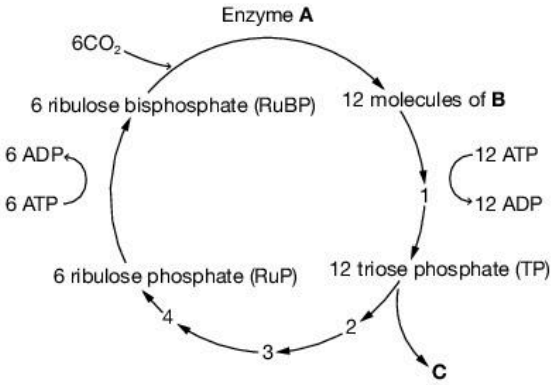
Temperature is a limiting factor of photosynthesis. The activity of the enzymes in chloroplasts will be much less ✔ so less ATP and reduced NADP needs to be produced by the light dependent stage, so the rate of photolysis decreases and less oxygen is produced. ✔ When light intensity is not limiting (50 mm and 100 mm) the rate of photosynthesis at 30 °C is about double that at 20 °C. This suggests that the reason is due to enzyme activity as $Q_{10} = 2$. ✔

There is good use of terminology in this answer that again is awarded full marks. Q_{10} is the temperature coefficient.

Exam-style questions

Structured questions (Paper 4)

1 The figure shows the Calvin cycle.



(a) (i) Name enzyme **A**, substance **B** and one of the substances formed at **C**. [3]

(ii) State the source of ATP. [1]

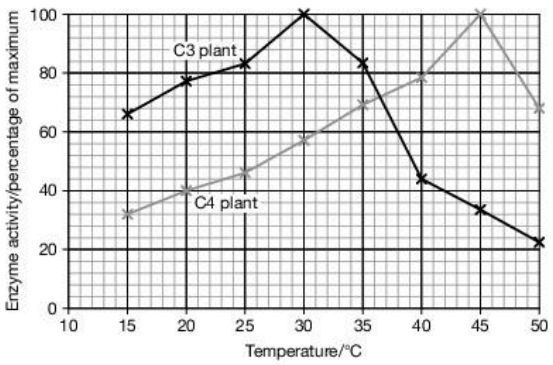
(iii) Name the precise site of the reactions of the Calvin cycle. [1]

(iv) State the number of carbon atoms in ribulose bisphosphate. [1]

(b) Explain the advantage of the reactions forming a cycle, as shown in the diagram. [2]

(c) Discuss the roles of pigments and electron carriers in photosynthesis. [6]

2 Phosphoenolpyruvate carboxylase (PEPC) is an enzyme that catalyses the initial fixation of carbon in C4 plants. In C3 plants it carries out other roles associated with carbohydrate metabolism. Researchers in India investigated the activity of PEPC from *Amaranthus hypochondriacus*, a C4 plant, and *Pisum sativum*, a C3 plant, at different temperatures. Their results are shown in the graph.



(a) Compare the effect of temperature on the PEPC from the C4 plant with the same enzyme from the C3 plant. [4]

Sorghum bicolor is a C4 crop plant. The leaves of *S. bicolor* have two cell types that carry out photosynthesis:

- mesophyll cells
- bundle sheath cells

Some of the differences between these two cell types are shown in the table.

Feature	Mesophyll cells	Bundle sheath cells
phosphoenolcarboxylase (PEPC)	present	absent
rubisco (ribulose bisphosphate carboxylase oxygenase)	absent	present
grana in chloroplasts	present	few

(b) Explain the differences between mesophyll cells and bundle sheath cells as shown in the table. [6]

Free response questions (Paper 4)

3 (a) Describe the formation of ATP in a palisade mesophyll cell during the daylight hours. [10]

(b) Discuss the importance of ATP in plant cells. [5]

4 (a) Describe how a chloroplast is adapted to carry out photosynthesis efficiently. [9]

(b) Explain how knowledge of limiting factors of photosynthesis is applied to increase the production of crop plants in protected environments. [6]

extra questions available online

14 Homeostasis

Key points

- ☐ Discuss the importance of homeostasis in mammals and explain the principles of homeostasis in terms of internal and external stimuli, receptors, central control, co-ordination systems and effectors (muscles and glands).
- ☐ Define the term negative feedback and explain how it is involved in homeostatic mechanisms.
- ☐ Outline the roles of the nervous system and the endocrine system in co-ordinating homeostatic mechanisms.
- ☐ Describe the deamination of amino acids and outline the formation of urea in the urea cycle.
- ☐ Describe the gross structure of the kidney and the detailed structure of the nephron with its associated blood vessels.
- ☐ Describe how the processes of ultrafiltration and selective reabsorption are involved with the formation of urine in the nephron.
- ☐ Describe the roles of the hypothalamus, posterior pituitary, ADH and collecting ducts in osmoregulation.
- ☐ Explain how the blood glucose concentration is regulated.
- ☐ Outline the role of cyclic AMP as a second messenger with reference to the stimulation of liver cells by adrenaline and glucagon.
- ☐ Describe the three main stages of cell signalling in the control of blood glucose by adrenaline.
- ☐ Explain how urine analysis is used in diagnosis with reference to glucose, protein and ketones including the principles of operation of dip sticks and of biosensors.
- ☐ Describe the structure and function of guard cells and explain the mechanism by which they open and close stomata, and regulate water loss by transpiration.
- ☐ Describe the role of abscisic acid in the closure of stomata during times of water stress.
- ☐ Outline the role of calcium ions as a second messenger in plants.

The importance of homeostasis

In a multicellular organism, the actions of different specialised cells need to be co-ordinated by the nervous system and the endocrine system. Various dynamic mechanisms keep conditions within cells constant.

Homeostasis is the maintenance of physiological conditions inside the body at a near constant level despite changes in the environment and in the body.

Thermoregulation

Thermoregulation is an example of a homeostatic mechanism. Mammals have complex physiological methods that not only control their core body temperature, but also maintain this temperature above (or slightly below) that of their surroundings and keep it constant (Figure 14.1).

Excretion

Excretion is the removal from the body of toxic substances, the waste products of metabolism and substances that are in excess of requirements. All substances that are excreted have passed through the blood at some stage.

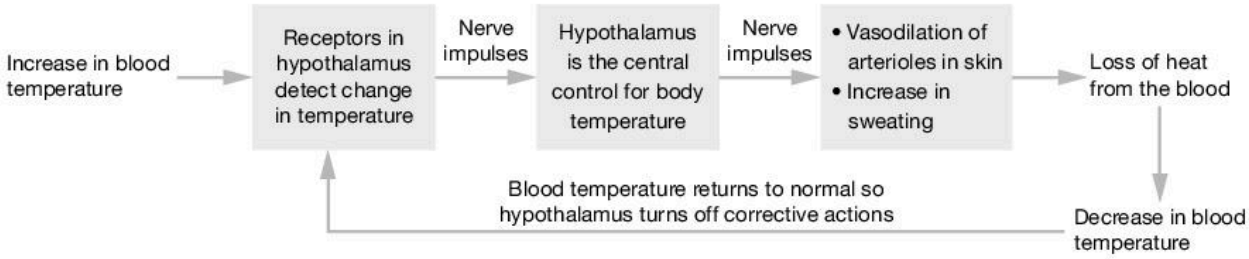
Exam tip

Thermoregulation is a good example to show the principles of homeostasis. You should be prepared for questions in Paper 4 that use unfamiliar examples to test the principles of homeostasis (see Raise your grade Question 1, page 168).

Link

This is a good opportunity to revise the effect of temperature and pH on enzyme activity (Unit 3).

A Level



▲ **Figure 14.1** This feedback loop shows what happens when the temperature of the blood increases, for example during exercise

Urea production

Excess amino acids are a good source of energy but the $-NH_2$ group must be removed by **deamination** before they can be respired. This $-NH_2$ group immediately forms ammonia, which is highly toxic. To excrete it safely requires huge volumes of water, so it is converted to urea, which is less toxic. The conversion of ammonia to urea happens in a cycle of reactions that occurs partly in mitochondria and partly in the cytosol of liver cells.

Key term

Deamination: the breakdown of an amino acid to remove the amino group ($-NH_2$).

Kidneys are the main excretory organ of urea

The kidneys are a pair of bean-shaped organs situated at the back of the abdominal cavity just below the diaphragm (see Figures 14.2 and 14.3).

▼ **Table 14.1** The regions of the kidney and their functions

Region of the kidney	Structural features	Function
capsule	thin layer of connective tissue with collagen fibres	protection
cortex	pale outer region glomeruli, proximal and distal convoluted tubules, blood vessels	ultrafiltration in glomeruli; selective reabsorption in proximal and distal convoluted tubules
medulla	dark inner region loops of Henle and collecting ducts with associated blood vessels (vasa recta)	formation of tissue fluid with a low water potential; reabsorption of water from loops of Henle and collecting ducts by osmosis to conserve water
pelvis	funnel-shaped space surrounded by white fibrous tissue	movement of urine from collecting ducts towards ureter
ureter	thick walled, muscular tube (smooth muscle)	movement of urine by peristalsis to bladder

The nephron

Kidneys are full of nephrons, each of which carries out all the processes necessary to make urine. Each nephron has a tube lined by a single layer of epithelial cells. The features of the different regions of the nephron can be seen with the light microscope:

★ Exam tip

When you write about homeostasis, use the terms listed here: **set point**, narrow limits, **stimulus**, **receptor**, monitor, control centre, **effector**, **negative feedback** and **corrective action**.

Key terms

Set point: the value for a physiological factor maintained as part of homeostatic equilibrium.

Stimulus: any change in the environment or inside the body that leads to a response.

Receptor: a cell or sensory neurone acting as a transducer to convert the energy of a stimulus into electrical impulses that travel along a neurone.

Effector: muscle or gland that carries out an action when stimulated by a nerve impulse or hormone.

Negative feedback: control mechanism that always returns a physiological factor to its set point to maintain homeostatic equilibrium.

Corrective action: a change in the body that restores the value for a physiological factor to its set point.

Search online for scanning electron micrographs of nephrons, especially the glomerulus, so that you can see how it is adapted for ultrafiltration.

- Glomerulus – a bundle of capillaries; surrounding the capillaries are podocytes - large cells with prominent nuclei (Figure 14.4).
- Bowman's capsule - a space surrounding each glomerulus where filtrate collects; the capsule is surrounded by a squamous epithelium.
- Proximal convoluted tubules (PCT) – formed of cuboidal epithelial cells, which have many microvilli.
- Loops of Henle – thin sections of loops are formed of squamous epithelium; thick sections of loops have thicker, cuboidal epithelium. Cells have no microvilli.
- Distal convoluted tubules (DCT) – formed of cuboidal epithelium without microvilli.

Nephrons drain into collecting ducts (CD). These are wider tubes formed of cuboidal epithelium without microvilli.

Excretion in the kidneys in mammals operates on the principle that everything below a certain size is filtered from the blood. This includes the waste products that are to be excreted and useful substances such as glucose, ions and amino acids. The useful substances are taken back into the blood by **selective reabsorption**. The rest are excreted.

Ultrafiltration

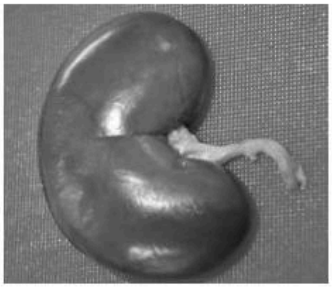
Blood at high pressure flows into the glomerulus, which is adapted for efficient **ultrafiltration**.

- Blood pressure is high as the renal artery branches off from the aorta close to the heart. The efferent arterioles have a smaller diameter than the afferent arterioles, which builds up pressure in the glomerulus.
- The capillaries have numerous endothelial pores in their walls which are at least 4 nm in diameter.
- Around each capillary is a basement membrane made of fibrous proteins that acts like a mesh to allow everything with a relative molecular mass of less than 69 000 through into Bowman's capsule.
- The glomerular capillaries are suspended within Bowman's capsule by podocytes, cells with projections that do not form a complete lining around the capillaries. The foot-like processes of different cells slot between each other to form gaps known as slit pores (Figure 14.4).

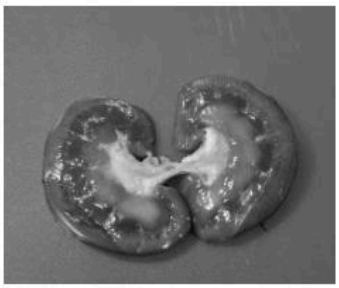
Selective reabsorption

Most of the filtrate is reabsorbed in the proximal convoluted tubule (PCT). The cells of this region of the tubule are adapted for movement of substances from the filtrate to the blood (Figure 14.5). The surface membrane lining the lumen of the PCT has microvilli to increase the surface area for absorption. Sodium-potassium pump proteins in the lateral and basal membranes move sodium ions against their concentration gradient into the tissue fluid. This provides the concentration gradient for absorption of sodium ions *and* glucose at the luminal surface. The sodium-potassium pump proteins require ATP, which is supplied by the many mitochondria in each cell.

Selective reabsorption involves movement through the PCT cells. The cells are attached together by tight junctions around the upper part of the cells. These are like 'sticky strips' that hold the cells together and prevent movement of fluid between cells from the lumen of the PCT into the blood. Reabsorption continues until all the glucose is removed from the filtrate.



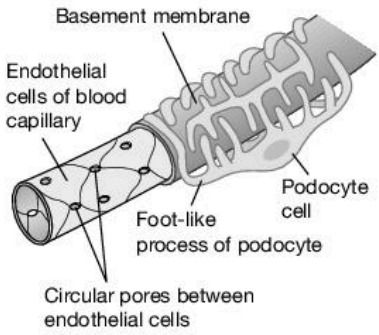
▲ **Figure 14.2** External view of a kidney showing the renal artery, renal vein and ureter dissected from the surrounding connective tissue and fat tissue



▲ **Figure 14.3** An internal view of the kidney showing the thin capsule, cortex, medulla, pelvis and ureter

Exam tip

Make a labelled diagram of a nephron from memory. You can use it to answer a free response question on the structure and function of the kidney. Make vertical and horizontal sections through a kidney – use the internet for guides on how to do this – and compare them with the microscope slides of kidney tissue and drawings to interpret what you see.



▲ **Figure 14.4** A small part of a capillary in a glomerulus with surrounding podocytes

The luminal membranes of PCT cells also have carrier proteins for the reabsorption of amino acids. Urea diffuses easily through cell membranes, so about 50% of urea in the filtrate moves across the cells of the PCT back into the blood by simple diffusion. The movement of the solutes into the PCT cells and into the blood creates a water potential gradient and water moves from the filtrate by osmosis back into the blood.

As the filtrate moves down the descending limb and up the ascending limb of the loop, it gets first more concentrated and then less concentrated. The filtrate that passes from the end of the loop to the DCT is less concentrated than that which enters the loop from the PCT as most of the ions have been pumped out.

The role of the loops is to provide a concentrated solution in the tissue fluid in the medulla. This highly concentrated tissue fluid surrounds the collecting ducts and is used in the reabsorption of water from the urine in the collecting duct/DCT. The upper part of the ascending limb is impermeable to water.

Osmoregulation

The kidney is the effector in **osmoregulation**. The kidneys filter a large volume of water. Much of this is reabsorbed to prevent the tissues becoming dangerously dehydrated. Urine contains water to dissolve the solutes, such as urea and excess sodium ions, that we need to remove. The volume of water lost in the urine can be controlled by reabsorbing more water when the body is dehydrated compared to when there is sufficient water in the body.

Role of the hypothalamus in osmoregulation

The hypothalamus has special nerve cells known as **osmoreceptors** that monitor the water potential of the blood, so it is kept within narrow limits. When the water potential decreases *below* the set point because too much water has been lost from the blood, osmoreceptors activate neurosecretory cells in the hypothalamus that synthesise the peptide hormone ADH. ADH is transported down their axons, which end in the posterior pituitary gland. Impulses are sent down the axon to cause the exocytosis of ADH into the blood (neurosecretion).

ADH binds to receptors on the cell surface membranes of the DCT and CD cells leading to the movement of vesicles containing aquaporins. The effect of ADH on its target cells involves the second messenger, cyclic AMP. The vesicles move towards the luminal membranes of the epithelial cells where they fuse with the cell surface membrane. The luminal membrane is impermeable until aquaporins are inserted. Other aquaporins are always in the basal and lateral membranes of the epithelial cells to let water move out of the cells into surrounding tissue fluid and into the blood. The solutes that contribute to the low water potential in the medulla are sodium ions, chloride ions and urea.

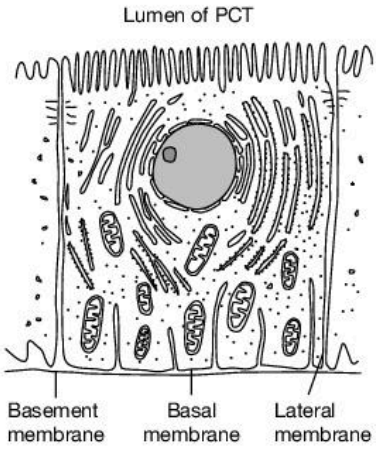
When water potential is restored to the set point, ADH secretion stops. ADH has a short half-life (10–30 minutes) so its concentration in the blood decreases quickly. Without the stimulation by ADH, the DCT and CD cells remove the aquaporins from the luminal surface and the permeability to water of these cells decreases. Water is not reabsorbed so a dilute urine is produced. This removes excess water from the body.

This method of control is **negative feedback** as the water potential of the blood is kept within narrow limits and any slight deviation from the set point triggers events that return the water potential to normal.

Key terms

Selective reabsorption: movement of certain substances from the glomerular filtrate into the blood by diffusion and active transport.

Ultrafiltration: movement of small molecules through the basement membrane surrounding capillaries in the glomerulus under high pressure.



▲ **Figure 14.5** A cell from the proximal convoluted tubule showing how it is adapted for selective reabsorption

Key term

Osmoregulation: the control of the water potential of the blood.

★ Exam tip

The 'concentration' of the body fluids is rarely given in text books and web sites as a water potential. You may find osmotic potential, osmotic pressure, osmolarity and osmolality. At A-level your response should be in terms of water potential (see Unit 4 pages 44–45).

The half-life of a hormone is the time taken for its concentration in the blood to decrease by a half.

Cell signalling

Communication between cells is chemical and electrical. Cells release chemicals to influence the activity of other adjacent and distant cells. This tends to be rather a slow way to communicate, although quite adequate for some functions. Neurones are specialised cells that send electrical impulses, often over long distances, allowing fast communication.

Some hormones are water soluble and cannot enter their target cells. There are receptors on the surface of these cells specifically for each of these hormones. Adrenaline acts at the cell surface membrane to stimulate the formation of cAMP, which is a second messenger. cAMP activates the first of several enzymes in a cascade that results in enzymes becoming active and breaking down glycogen to glucose.

Oestrogen and progesterone are steroid hormones that are not water soluble. These hormones pass through the phospholipid bilayer, interact with receptors inside the cytoplasm and activate transcription of certain genes.

Link

This section is about chemical communication using hormones. Communication by neurones is in Unit 15.

Link

Calcium ions are second messengers in many processes in plants and animals. See page 167 and Unit 15 pages 174–176 for examples.

Regulation of blood glucose concentration

The glucose concentration in the blood fluctuates but is kept within narrow limits. The concentration is usually within the range 80–120 mg glucose per 100 cm^{-3} blood and is normally about 90 mg 100 cm^{-3}. If it rises too high, then the kidney cannot reabsorb all the glucose that is filtered from the blood and some will be lost from the body in the urine. This happens if the concentration of glucose in the blood is above 180 mg 100 cm^{-3}, which is known as the **renal threshold**. Glucose lost in the urine is obviously not respired or stored as glycogen or as fat. If the glucose concentration falls too low, then there is not enough for the brain cells and a person may enter a coma. This happens at concentrations less than 60 mg 100 cm^{-3}.

The following occur when the concentration *increases* above the set point.

1. The increasing concentration of glucose acts as a stimulus.
2. β cells in the islets of Langerhans (Figure 14.6) secrete insulin in response. (α cells stop releasing glucagon so glucose is not released into the blood by liver cells at the same time.) Insulin circulates in the blood stream and binds to insulin receptors on target cells: liver cells, adipose cells and muscle cells.

Insulin has a number of effects on **liver cells**:

- stimulates the increased uptake of glucose
- stimulates the conversion of glucose into glycogen (glycogenesis) by activating the enzyme glycogen synthase
- inhibits the enzyme glycogen phosphorylase that breaks down glycogen to glucose
- inhibits the conversion of fats and proteins into glucose

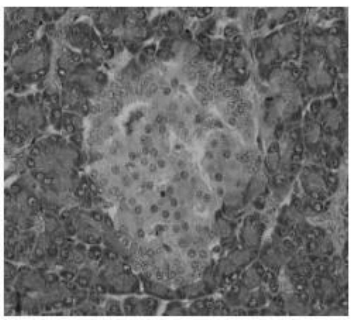
Insulin stimulates **muscle cells** and **adipose cells** to insert more glucose transporter proteins (GLUT proteins) into their cell surface membranes to increase the uptake of glucose. The enzymes in muscle cells that convert glucose to glycogen are activated. The enzymes that convert glucose to fat are activated in adipose cells.

When the concentration *decreases* below the set point, these changes occur.

1. α cells respond to decreasing concentrations of glucose by releasing glucagon. They also stimulate β cells to stop secreting insulin.

Exam tip

You can show the fluctuations that occur in blood glucose by drawing a sine wave on a pair of axes. The horizontal axis is time and the vertical axis is blood glucose concentration per 100 cm^{-3} blood. As you read this section, relate what happens to the increases and decreases shown on your graph. Add the figures from this paragraph to the vertical axis.



▲ Figure 14.6 Photomicrograph of the pancreas: light tissue is an islet of Langerhans with α cells and β cells; dark surrounding tissue produces enzymes that are secreted through the pancreatic duct into the small intestine

2. Glucagon circulates in the bloodstream and binds to glucagon receptors on liver cells.
3. The receptors interact with other membrane proteins to increase the concentration of cyclic AMP inside liver cells.

Glucagon has these effects on liver cells:

- stimulates **glycogenolysis** (literally: splitting glycogen) by activating the enzymes that breakdown glycogen
- stimulates the conversion of fat and protein into intermediate metabolites that are converted into glucose – **gluconeogenesis**.

These two processes lead to an increase in the concentration of glucose in liver cells, so glucose diffuses out into the blood. The concentration of glucose in the blood increases and this keeps cells supplied with their main source of energy.

Key terms

Glycogenesis: synthesis of glycogen from glucose to decrease blood glucose concentration when stimulated by insulin.

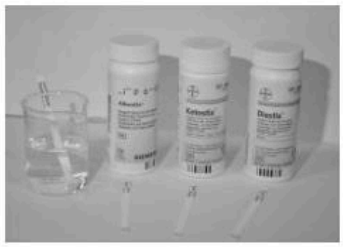
Glycogenolysis: breakdown of glycogen to form glucose to increase blood glucose concentration when stimulated by glucagon.

Gluconeogenesis: formation of glucose from non-carbohydrate sources, such as amino acids.

Dip sticks and biosensors

Dip sticks and biosensors use biological agents such as enzymes and antibodies to detect specific chemicals.

- A dip stick is a plastic strip with a pad at one end impregnated with the biological agents. The colour of the pad changes if the chemical is present. The user has to compare the colour of the pad to a colour chart to assess the quantity present.
- A biosensor uses biological agents to detect chemicals, but also has a transducer that converts the change brought about by the biological agent (a biological signal) to an electric signal that can be quantified to give a digital display.



▲ **Figure 14.7** Dip sticks used to test urine for albumen, ketones and glucose

Homeostasis in plants

Plants respond to changes in their environment to maintain internal conditions. Carbon dioxide concentrations inside leaves need to be maintained for photosynthesis. Opening stomata allows the diffusion of carbon dioxide into internal air spaces, but also allows water vapour to diffuse out. If the water cannot be replaced quickly enough because rates of transpiration are higher than rates of water absorption, plants often wilt. This moves the leaves out of the direct rays of the sun and is a homeostatic mechanism. A few cells at the base of the leaf become flaccid, lowering the leaf and reducing water loss. Stomata may close to reduce rates of transpiration.

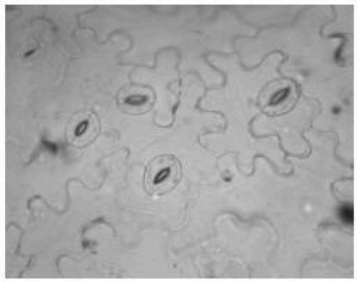
Link

Rates of transpiration are dependent on the width of stomatal pores, which is controlled by guard cells. This is a good opportunity to revise transport of water in plants and transpiration from Unit 7.

Stomata

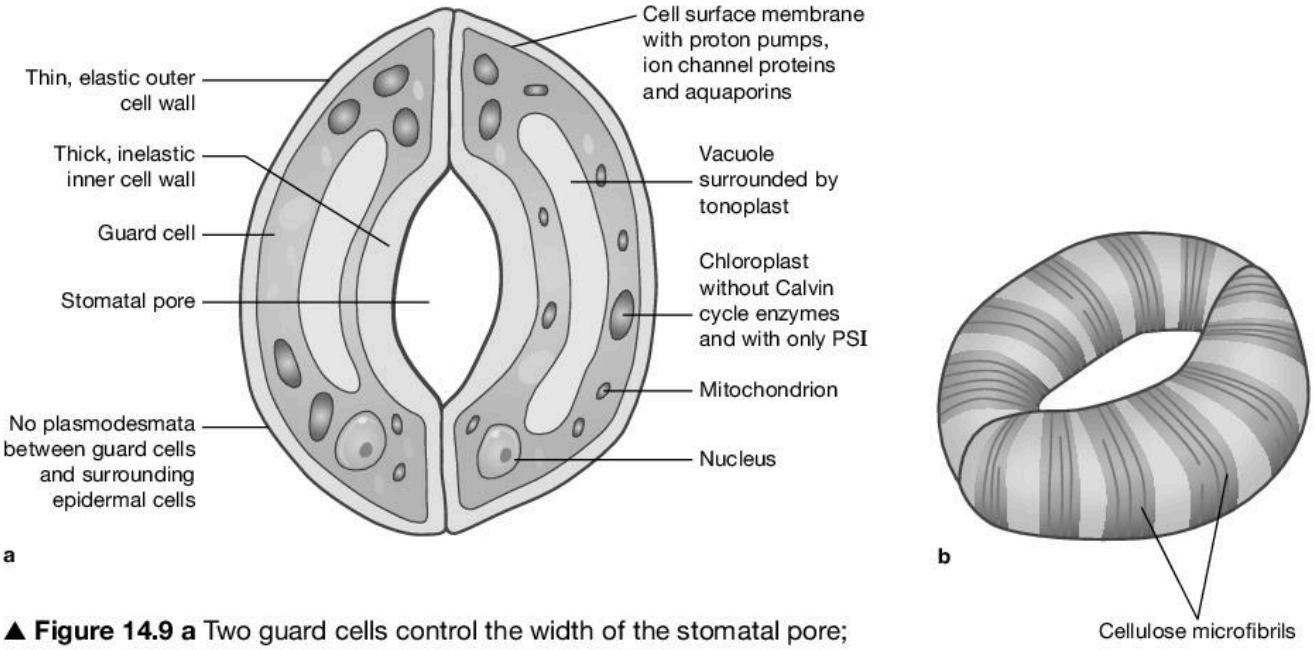
Guard cells are sensitive to light intensity, humidity, temperature and to carbon dioxide concentrations inside leaves. Stomata tend to be closed at night and open during the day, although there are some species where this rhythm is reversed.

Stomatal opening is dependent on the detection of light by pigment molecules in the cytoplasm of guard cells that respond by activating proton pumps in the cell surface membranes. These proton pumps move protons *out* of the cell using ATP as their source of energy. This makes the inside of the cell more negative, a change which stimulates potassium ion influx channel proteins to open. Potassium ions diffuse into the guard cells. Other ion channels open allowing the diffusion of anions (chloride and nitrate) to maintain electroneutrality. Starch is converted to malate, which increases the concentration of solutes in the guard cells, so decreasing the water potential.



▲ **Figure 14.8** Stomata on the lower epidermis of *Cymbalaria muralis* × approx. 500

Water enters the cell through aquaporins. The cells become turgid and expand. Cellulose microfibrils in the cell walls prevent guard cells becoming any wider. As each guard cell enlarges it lengthens, pushing against the other guard cell at either end; both cells bow outwards, so opening the pore between them (Figure 14.9).



▲ **Figure 14.9 a** Two guard cells control the width of the stomatal pore; **b** distribution of cellulose microfibrils around guard cells

In the dark, proton pumps stop functioning. The inside of the cell becomes less negatively charged and potassium ion influx channel proteins close. Other channel proteins open to allow potassium ions to diffuse out. Other ions also diffuse out and malate is converted back into starch. The decrease in solute concentration causes an increase in water potential in guard cells so that water flows out of the guard cells by osmosis. The cells become flaccid and the guard cells are pushed closer together by adjacent cells and the stomatal pore closes.

Abscisic acid and closure of stomata When water is in short supply, abscisic acid (ABA) is released by roots and by the chloroplasts of mesophyll cells. ABA stimulates guard cells to close, so reducing the rate of transpiration and helping to conserve water within the plant. The binding of ABA to receptors on guard cell membranes activates a cell signalling cascade that has the following effects:

- Diffusion of calcium ions into the cytosol.
- Calcium ions act as second messengers by activating ion channels in the cell surface membrane so that potassium ions and anions diffuse out.
- Inhibition of proton pumps that move hydrogen ions out of guard cells.

★ Exam tip

The pumping of protons out of the cell to stimulate the influx of ions is similar to the role of proton pumps in companion cells (see Unit 7 page 84).

Raise your grade

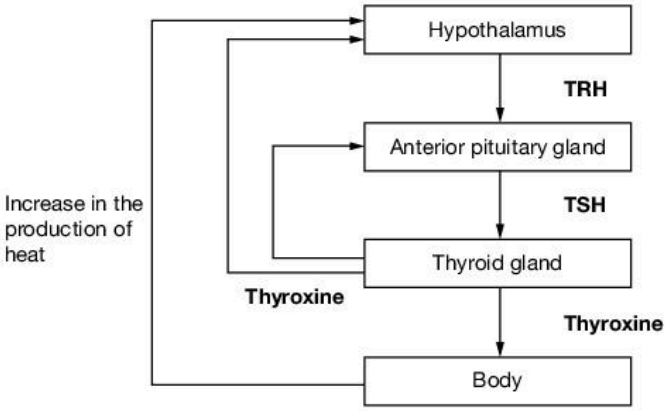
1 Thyroxine is a hormone secreted by the thyroid gland, which is an endocrine gland. One of the roles of thyroxine is to increase the production of heat in the body.

(a) Apart from synthesising and secreting hormones, state one feature that would confirm that the thyroid gland is an endocrine gland. [1]

The hormone is released directly into the blood, not into a duct and then to another organ. ✔

Correct answer.

The flow chart shows how the release of thyroxine is controlled by the release of hormones by the anterior pituitary gland and the hypothalamus.



Use the information above to answer the following questions.

(b) State what will happen to the secretion of thyroxine into the blood if:

(i) the secretion of TSH increases; [1]

Thyroxine secretion into the blood increases. ✔

Correct answer.

(ii) the secretion of TRH decreases; [1]

The release of TSH increases. ✘

The candidate has not read the question carefully and not looked at the diagram.

(iii) the production of heat in the body increases greatly. [1]

The thyroxine secretion decreases ✔ because the temperature is above the set point of 37°C.

This is correct but there is nothing in the diagram about temperature. The candidate did not need to include an explanation. Giving more information than required can sometimes lead to loss of marks as it is possible to give information that is biologically incorrect and contradicts the right answer.

(c) (i) State a target organ for thyroxine that will result in high heat production. [1]

Liver.

The liver is a target organ; most of the body's heat is produced by respiration in the liver and in muscle contraction.

(ii) The half-life of thyroxine is about seven days. Suggest how the term *half-life* is applied to hormones. [1]

The length of time a hormone is in the body; it's the time taken for the concentration to decrease by a half assuming no more is secreted.

Correct answer.

(d) TRH is a neuropeptide. Explain what this means. [2]

It is a peptide hormone made of a few amino acids joined by peptide bonds. ✔

The candidate has not explained the 'neuro' part of the term. TRH is released by modified neurones (which are in the hypothalamus).

(e) With reference **only** to information in the diagram, explain how negative feedback is involved in the secretion of thyroxine. [3]

There is a normal concentration of thyroxine in the blood. If the body gets too hot, then less thyroxine is needed to stimulate heat production so less is released by the thyroid gland. ✔ This is because the hypothalamus detects the blood temperature and releases less TRH ✔ so the pituitary releases less TSH. ✔ If it gets too cold the opposite things happen.

The candidate has given an adequate answer explaining how the concentration of thyroxine is reduced when the body overheats.

2 Selective reabsorption occurs in the proximal convoluted tubule of each nephron in the kidney.

(a) Explain how cells in the proximal convoluted tubule are adapted for selective reabsorption. [5]

These cells have many microvilli that give a large surface area for absorbing substances from the filtrate in the proximal convoluted tubule. ✔ Some of these like glucose and sodium are absorbed by active transport. This needs ATP which is supplied by the many mitochondria ✔ in the cells. Mitochondria have many cristae to give large surface area for the protein carriers in the ETC and for many ATP synthase molecules. The membranes of PCT cells have many co-transporters for sodium and glucose.

This is *not* a well-structured answer. The first sentence clearly links structure to function and the point about many mitochondria gains a mark. The adaptations of mitochondria are not relevant as they apply to all cells with high demands for ATP. The last sentence does not state that it is the *luminal* membranes that are the location of the co-transporters. Sodium *ions* are present in body fluids and in cells, not sodium.

(b) Explain the changes that occur to the volume and composition of the glomerular filtrate as it flows through the proximal convoluted tubule. [5]

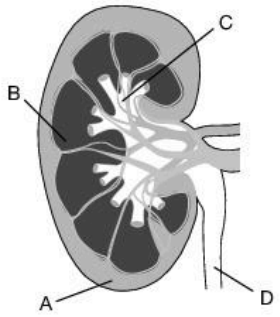
The volume of filtrate decreases as most of the water is reabsorbed down a water potential gradient by osmosis into the blood. ✔ This happens because solutes are reabsorbed by active transport. All the glucose ✔ and amino acids ✔ and most of the sodium ions ✔ are reabsorbed too by carrier proteins in the membranes of the cells. This changes the composition of the filtrate so that it has no glucose and no amino acids and much less sodium ions. Urea diffuses back in the blood so the filtrate has less urea. ✔

All correct. Notice that it is necessary to include some description: 'volume … decreases' and 'changes composition…' even though the question says 'Explain'. In questions like this when asked to explain, keep any descriptions as short as necessary for the answer.

Exam-style questions

Structured questions (Paper 4)

1 The kidneys are one of the main excretory organs of the body. The diagram shows a vertical section of a kidney.

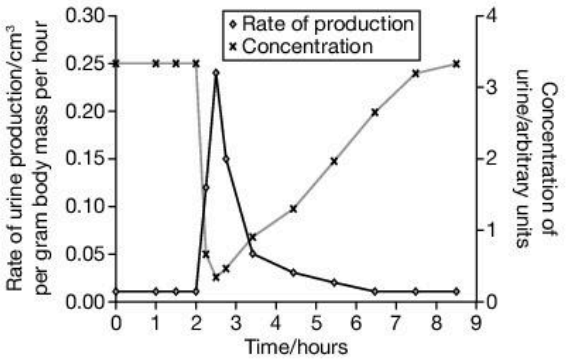


(a) Identify the parts of the kidney labelled **A** to **D**. [4]

(b) Describe briefly the functions of the parts labelled **C** and **D**. [2]

2 The common vampire bat, *Desmodus rotundus*, is found in Trinidad and Central America. This bat feeds on the blood of sleeping mammals ingesting about 60% of its body mass in blood with each meal. This protein-rich food has the same water potential as the bat's blood plasma but has a high volume. The stomachs of vampire bats concentrate the blood meals very quickly by absorbing water.

The rate of urine production and concentration of urine produced by a captive common vampire bat was determined before and after one blood meal. The bat was provided with a blood meal during the second hour of the investigation. The results are shown in the graph.



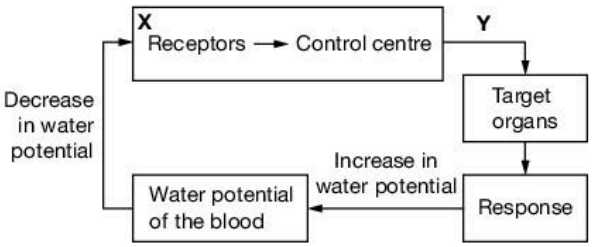
(a) Describe the immediate effect of feeding on the the rate of production of urine and the concentration of urine. [4]

(b) Explain the benefits to the bat of the effects you have described in **(a)**. [3]

(c) Explain why *D. rotundus* excretes large quantities of urea. [3]

(d) Vampire bats are able to produce a much more concentrated urine than that produced by humans. Suggest how they are able to do this. [2]

3 The water potential of the blood of mammals is maintained within narrow limits. The flow chart below shows how the water potential of the blood is controlled when it decreases.



(a) Name

(i) the part of the brain shown by box **X**, [1]

(ii) the hormone shown by **Y**, [1]

(iii) the target organs. [1]

(b) Describe the response carried out by the target organs to increase the water potential of the blood. [3]

(c) Use this example to explain how negative feedback is used to maintain constant conditions in the body. [5]

Free response questions (Paper 4)

4 (a) Outline the different ways in which cells send signals to each other. [9]

(b) Describe the role of the liver in the control of blood glucose. [6]

5 (a) Describe the structure of guard cells and explain the mechanism by which they open stomata. [9]

(b) Abscisic acid (ABA) is produced by plants when there is a shortage of water. Explain how ABA stimulates the closing of stomata. [6]

extra questions available online

15 Control and co-ordination

Key points

- ☐ Describe the structure of a sensory neurone and a motor neurone.
- ☐ Outline the roles of sensory receptor cells in detecting stimuli and stimulating the transmission of nerve impulses in sensory neurones.
- ☐ Describe the functions of sensory, relay and motor neurones in a reflex arc.
- ☐ Describe and explain the transmission of an action potential in a myelinated neurone and its initiation from a resting potential.
- ☐ Explain the role of sodium ions and potassium ions in the transmission of action potentials.
- ☐ Explain the importance of the myelin sheath (saltatory conduction) in determining the speed of nerve impulses and the refractory period in determining their frequency.
- ☐ Describe the structure of a cholinergic synapse and explain how it functions.
- ☐ Outline the roles of synapses in the nervous system.
- ☐ Describe the ultrastructure of striated muscle with particular reference to sarcomere structure.
- ☐ Describe the roles of neuromuscular junctions, transverse system tubules and sarcoplasmic reticulum in stimulating contraction in striated muscle.
- ☐ Explain the sliding filament model of muscular contraction.
- ☐ Explain the roles of the hormones FSH, LH, oestrogen and progesterone in controlling changes in the ovary and uterus during the human menstrual cycle.
- ☐ Outline the biological basis of contraceptive pills containing oestrogen and/or progesterone.
- ☐ Compare the nervous and endocrine systems as communication systems that co-ordinate responses to changes in the internal and external environment.
- ☐ Describe the role of gibberellin in the germination of wheat or barley and explain its role in stem elongation.
- ☐ Explain the role of auxin in elongation growth.
- ☐ Describe the rapid response of the Venus fly trap and explain how the closure of the trap is achieved.

Neurones

Nerve cells are found in both parts of the nervous system, the central nervous system (CNS), which is divided into the brain and spinal cord, and the peripheral nervous system (PNS) which consists of nerves – cranial nerves attached to the brain and spinal nerves attached to the spinal cord.

Nerve cells, known as **neurones**, transmit information very fast over long distances.

There are three types of neurone: **sensory neurone, relay neurone** and **motor neurone**.

Sensory neurones:

- transmit impulses from sensory cells (receptor cells) or sensory dendrites to the CNS
- have their cell bodies in ganglia – swellings on nerves just outside the CNS
- synapse with motor or relay neurones within the CNS.

★ Exam tip

Take care over using the terms nerve and neurone. A nerve contains many neurones surrounded by protective fibrous tissue, e.g. optic nerve.

★ Exam tip

Make drawings of the three types of neurone, label them and add annotations explaining the functions of the different parts. This will make a good resource for your revision.

A Level

Motor neurones:

- transmit impulses from the CNS to effectors – muscles and glands
- have their cell bodies within the CNS or in ganglia.

Relay neurones:

- transmit impulses from sensory to motor neurones
- are found entirely within the CNS.

Sensory receptors

Sensory receptors are **transducers** that convert the energy of a stimulus into electrical impulses. Stimulation of a receptor leads to a receptor potential – a change in the potential difference across the cell surface membrane. If this is great enough, the receptor causes impulses to travel along sensory neurones. Some receptors detect changes in the external environment: chemoreceptors in the nose and tongue have receptor molecules on their cell surface membranes that have shapes complementary to those of the molecules that they detect. Receptors detect changes inside the body; for example, muscle spindles detect the degree of tension in muscle tissue and receptors in the hypothalamus detect changes in the temperature of the blood.

Link

See Question 4 on page 254 for more information on the role of chemoreceptors.

Link

There is more information on initiation of action potentials and transmission of nerve impulses later in this Unit.

Neurones function in reflex arcs

The simplest pathway involving neurones is a reflex arc, which usually consists of a sensory neurone, a relay neurone and a motor neurone. In the knee jerk reflex, the pathway has no relay neurone. Neurone pathways do not have to pass through the brain. The reflex arc that controls the withdrawal of the hand from hot or sharp objects passes through the spinal cord and not through the brain.

Key term

Reflex arc: the neural pathway involving the sensory receptor, sensory neurone, relay neurone and motor neurone that detects a stimulus and coordinates the response by an effector.

Myelinated neurones

Myelinated neurones are supported by Schwann cells that grow around the axons until there are many layers of cell membrane that form **myelin**. These membranes are rich in phospholipids, with very few proteins. Myelin insulates axons from tissue fluid as ions cannot diffuse through the layers of phospholipids. The spaces between adjacent Schwann cells lack myelin and form gaps – **nodes of Ranvier** – that allow tissue fluid to reach the surface of axons.

Exam tip

Remember that current flows in axons longitudinally (along the axon), but during action potentials ions flow transversely (in and out) of the axon to boost current flow.

Structure of the cell surface membrane of axons

Axon membranes have many transport proteins for the movement of sodium ions and potassium ions. Active transport occurs using sodium–potassium pumps, which act continually to transport the ions across the membrane (as in almost all other cells). Passive transport occurs through two main types of channel protein: voltage-gated channels and ion-leak channels.

Voltage-gated channel proteins alternate between being open allowing ion flow, being closed and unable to open; or being closed and able to open. They respond to changes in the potential difference across the membrane. The transport proteins are found along the whole length of unmyelinated neurones but are concentrated at nodes in myelinated neurones.

The nerve impulse

Conduction of nerve impulses relies on passive current flow along axons. The resistance to current flow is high and impulses decay quickly. To 'boost' current flow, action potentials occur at intervals. An action potential is the net effect of ion flow *across* the axon membrane. In unmyelinated neurones

Key term

Action potential: a rapid and brief reverse in the potential difference across a membrane that acts to boost current flow in neurones and muscular tissue.

action potentials occur all along the axons. In myelinated neurones, action potentials occur only at the nodes of Ranvier.

Resting potential

The inside of the neurone is negatively charged with respect to the outside, so the **resting potential** is usually written as −65 mV or −70 mV.

The resting potential is the result of an unequal distribution of ions across membranes. The factors that contribute to a resting potential of a neurone are:

- the presence of many organic anions inside the cell, such as negatively charged proteins
- carrier proteins pump out three sodium ions for every two potassium ions pumped in (sodium–potassium pumps)
- the impermeability of the membrane to ions. The phospholipid bilayer has a hydrophobic core which does not permit the movement of ions
- voltage-gated channel proteins are shut so sodium and potassium ions cannot diffuse through them
- the leakage of some potassium ions through the potassium leak channel proteins. As the inside of the cell is negatively charged this attracts potassium ions so few, in fact, diffuse out through these channels.
- the limited diffusion of sodium ions into the neurone when it is at the resting potential, as there are there are very few leak channel proteins for sodium ions.

To maintain the sodium–potassium concentration gradients, the cell continually uses ATP.

The threshold potential is the potential difference across a membrane above which an impulse is sent along a neurone. Neurones are either stimulated to send impulses or not; if a stimulus is below threshold they do not send any impulses. This is the all or nothing law.

Key terms

Resting potential: the potential difference across a cell surface membrane of –60 to –75 mV.

Depolarisation: a change in the potential difference across a membrane so it becomes *less* negatively charged inside.

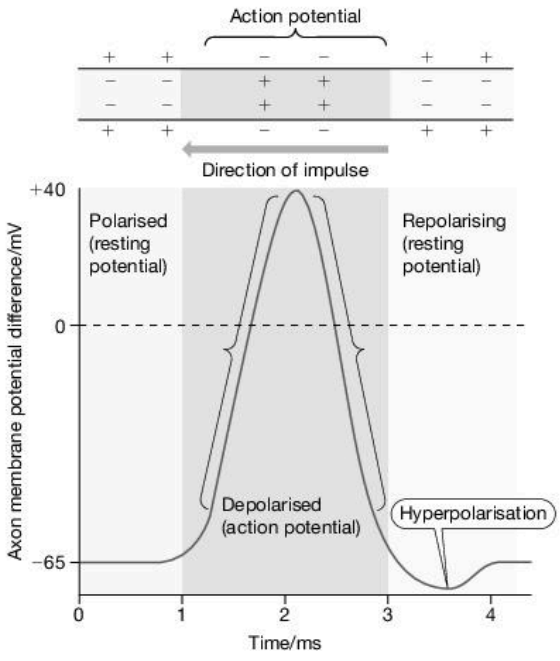
Hyperpolarisation: a decrease in the potential difference across a membrane by becoming *more* negatively charged inside.

Action potential

An action potential needs to occur to initiate an impulse (Figure 15.1). The membrane is depolarised and current flows forward along the neurone. This current flow **depolarises** the next region of membrane which triggers some of the voltage-gated channel proteins for sodium ions to open. Sodium ions diffuse into the axon down their electrochemical gradient and the membrane depolarises some more, which stimulates yet more channel proteins to open.

There is a **positive feedback** in which the depolarisation leads to more and more channel proteins opening to increase the amplitude of the depolarisation. The maximum potential difference is +30 to +40 mV and at this point voltage-gated sodium ion channels close and remain closed.

The increase in sodium ions reverses the charge inside the axon so that current flows onwards along the axon. This current flow depolarises the next patch of membrane which will be the next node in a myelinated neurone. Meanwhile the potential difference across the membrane needs to be restored to the original resting potential so that another action potential can occur. The resting potential is restored by the opening of voltage-gated potassium ion channels and the diffusion of potassium ions out of the axon. Voltage-gated sodium ion channels remain closed and cannot be stimulated to open.



▲ **Figure 15.1** An action potential. On an oscilloscope screen this appears as the upward stroke of the 'spike'

The period of time when the sodium channels do not respond to depolarisation is the **refractory period**. The overshoot in restoring resting potential is because more potassium ions diffused out than necessary to restore the resting potential. The refractory period separates action potentials and determines the maximum frequency of impulses (number of impulses per unit time) in a neurone. Repolarisation of the axon returns the axon to its original resting potential in readiness for a new stimulus if it comes. Sodium–potassium pumps function continually to maintain the concentrations of these two ions.

If you consider a patch of membrane in the middle of a neurone, an action potential causes current flow in both directions – towards the neurone terminal and towards the cell body. The refractory period is important to ensure that impulses travel only in one direction.

Synapses

Neurones are separate cells. The junction between two neurones is a **synapse**. The gap is called the **synaptic cleft**. Transmission across these synapses is chemical rather than electrical.

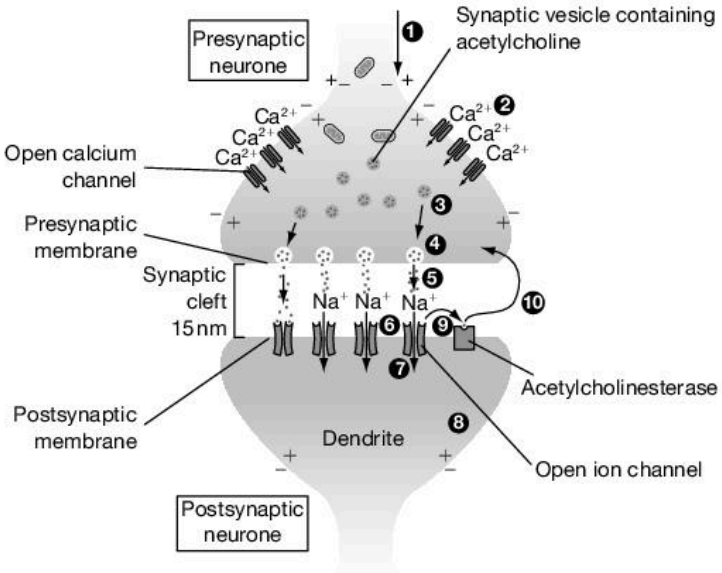
The target cells of neurones are either other neurones or, in the case of motor neurones, effectors (muscles and glands). Synapses between neurones are known as **interneuronal synapses** and those between motor neurones and muscle fibres as **neuromuscular junctions (motor end plates)**.

Synapses are categorised according to the **neurotransmitter** released. You need to know about **cholinergic synapses** in which **acetylcholine** is the neurotransmitter.

Cholinergic synapses

An axon terminal ends in a swelling called a synaptic bulb or bouton. The membrane here contains voltage-gated calcium ion channel proteins and the cytoplasm contains vesicles with acetylcholine (Figure 15.2).

The events that occur when an impulse arrives at a cholinergic synapse are summarised in the diagram and in steps 1 to 11 below.



▲ **Figure 15.2** Synaptic transmission. Follow the sequence of events described as 1 to 11 in the text

Exam tip

The length of time for the refractory period determines the highest frequency for action potentials in a neurone.

Exam tip

ACh is an accepted abbreviation for acetylcholine. But always write out the word in full first before using the abbreviation.

Key terms

Positive feedback: a control mechanism in which a change in a factor leads to an increase in that factor.

Refractory period: brief period of time following an action potential when a neurone cannot be stimulated.

Synapse: the junction between two neurones with a synaptic gap (also known as synaptic cleft) between them.

Neurotransmitter: the chemical released by the presynaptic neurone that diffuses across the synaptic gap to receptor proteins on the surface of the postsynaptic neurone.

Cholinergic synapse: the type of synapse which releases acetylcholine as the neurotransmitter.

1. An impulse arrives at the synaptic bulb.
2. In response to depolarisation, the voltage-gated channel proteins for calcium ions open.
3. Calcium ions diffuse into the cytoplasm down their electrochemical gradient. The concentration of calcium ions inside cytoplasm is very low (almost zero), so this is a steep gradient.
4. Calcium ions trigger the movement of vesicles along microtubules towards the presynaptic membrane.
5. The vesicles fuse with the presynaptic membrane and release their contents of acetylcholine molecules by exocytosis.
6. Acetylcholine molecules diffuse across the synaptic gap and combine with chemical-gated channel proteins on the postsynaptic membrane.
7. The channel proteins open to allow diffusion of sodium ions into the cytoplasm of the postsynaptic neurone down an electrochemical gradient.
8. The postsynaptic membrane is depolarised.
9. Acetylcholine molecules leave the protein channels and are broken down by the enzyme **acetylcholinesterase**. The products are acetyl groups and choline which diffuse back into the presynaptic membrane.
10. With removal of acetylcholine, the postsynaptic membrane is repolarised.
11. If the sum of all the impulses arriving at the postsynaptic neurone is greater than its threshold, then an impulse is sent.

Remember

Look back to Unit 4 to remind yourself about exocytosis. Remember that this involves the fusion of the membrane surrounding vesicles to the cell surface membrane.

Exam tip

Chemical-gated channel proteins are also known as ligand-gated channel proteins.

Remember

Neurotransmitters are cell signalling molecules although the distance they travel between neurones or between neurones and effector cells is very small.

Roles of synapses

Transmission of impulses. The main role of a synapse is to allow impulses to be transmitted from one neurone to another. They are directional as only the presynaptic membrane has vesicles of neurotransmitter and only the postsynaptic membrane has receptors.

Connection between one neurone and many others. The axons of many sensory and relay neurones divide into many branches. This allows an impulse from one neurone to stimulate many other others. Information can spread out from one sensory receptor to many areas within the central nervous system or from the CNS to many effectors. This is especially important in preparing the body to meet dangerous situations.

Exam tip

Make sure you are clear about the use of presynaptic and postsynaptic neurones.

Remember

The autoimmune disease myasthenia gravis is described on Unit 11 page 123. Recall that this disease is caused by the production of antibodies against proteins at synapses.

Exam tip

The nervous system and endocrine system work together in the 'fight or flight' response to danger. Nerves terminate in the adrenal glands and the arrival of impulses along neurones stimulate the release of adrenaline. Nerve impulses pass along neurones that form synapses with many others to coordinate rapid responses throughout the body.

Integration of impulses. Synapses allow integration of impulses from different neurones, both excitatory and inhibitory. Excitatory neurones release neurotransmitters that stimulate depolarisation in the postsynaptic neurone. The neurotransmitters released by inhibitory neurones stimulate ion flow that makes the inside of the neurone more negative. This is called **hyperpolarisation**. This decrease of the potential difference makes it more difficult to depolarise the neurone.

Link

The supply of ATP in striated muscle tissue is described in Unit 12 – see page 139.

Muscle contraction

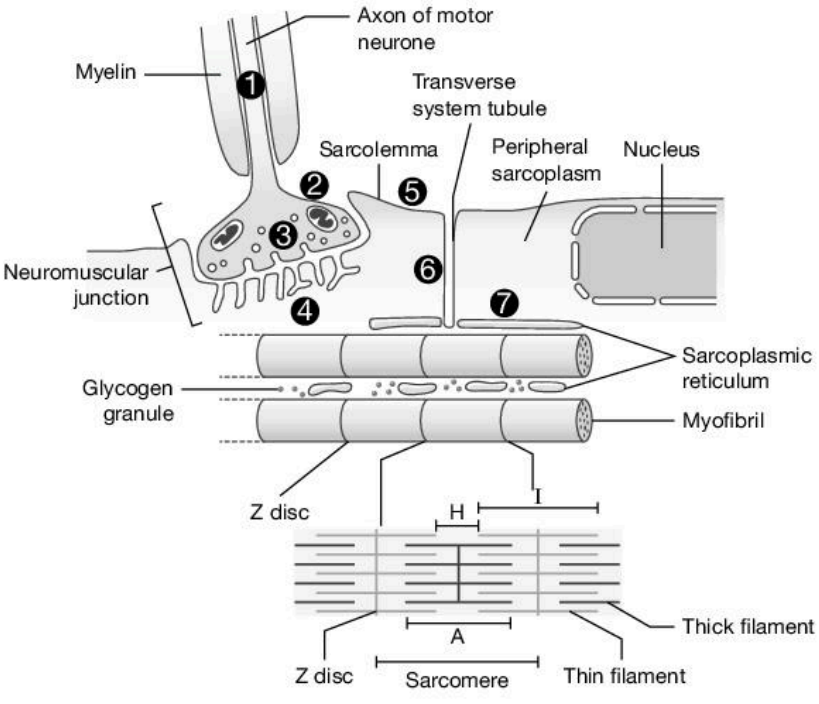
The main muscles of the body, such as those in arms and legs, are composed of **striated muscle tissue**. Each muscle contains many cylindrically shaped muscle fibres, each with many nuclei. Inside each muscle fibre are many **myofibrils**, each composed of **sarcomeres** (see Figure 15.3). The thin filaments are composed of the protein actin and the thick filaments are composed of myosin.

Motor neurones terminate at the motor end plate (neuromuscular junction). Action potentials travel down the motor neurone (1), stimulate voltage-gated channels to open allowing calcium ions to enter (2). Calcium ions cause vesicles of neurotransmitter to fuse with the membrane (3). Acetylcholine binds to receptors causing an inflow of sodium ions (4) that depolarise the **sarcolemma** (an end-plate potential). If above threshold, action potentials are transmitted across the sarcolemma (5) and down into **transverse (T) system tubules** (6). The sarcolemma and T-system tubules have the same channel proteins and pumps that are found in neurones.

The impulse is coupled to the contraction mechanism. Between the T-system tubules and the **sarcoplasmic reticulum** (SR) is a protein, which transfers the stimulus so that the sarcoplasmic reticulum is depolarised. Depolarisation of T-system tubules causes calcium ion channel proteins in the sarcoplasmic reticulum to open and release calcium ions into the sarcoplasm (7). These act as second messengers to stimulate movement of the muscle myofibrils.

This swivelling motion of myosin heads is the power stroke that moves the thin filaments closer together, reducing sarcomere length. The combined effect of shortening in all the sarcomeres shortens the length of the myofibril; in turn the combined effect of all the myofibrils in all the muscle fibres shortens the length of the muscle. When there is no action potential in the sarcolemma, calcium is pumped back into the sarcoplasmic reticulum and contraction stops.

The myosin head is an ATPase which hydrolyses ATP to ADP and P_i. When it moves during the power stroke ADP and P_i are released. ATP then binds to the myosin head that causes the myosin and actin to separate; the myosin head returns to its original position and is ready to repeat the process.



◀ **Figure 15.3** The sequence of events that occurs when nerve impulses arrive at the end of a motor neurone. The numbers refer to those in the text

Key terms

Striated muscle tissue: type of muscle tissue attached to the skeleton consisting of multinucleate fibres, each filled with many myofibrils.

Myofibril: cylindrical organelle found within muscle fibres. The parallel alignment of alternating dark and light bands of the myofibrils in a fibre gives the striated appearance.

Sarcomere: functional unit of myofibrils. Each sarcomere consists of thin filaments attached to Z lines at either end with thick filaments in between.

Sarcolemma: the surface membrane of each muscle fibre.

Transverse (T) system tubules: extensions of the sarcolemma that penetrate muscle fibres between the myofibrils.

Sarcoplasmic reticulum: modified endoplasmic reticulum that stores calcium ions. When activated it releases calcium ions by facilitated diffusion to stimulate the sliding of filaments in sarcomeres by binding to troponin.

★ **Exam tip**

Search for electron micrographs and diagram of striated muscle. Watch some animations to help your understanding of what happens when thick and thin filaments slide over each other.

Control and co-ordination in plants

Plant co-ordination primarily uses specific chemicals known as plant hormones. Plants do not have nervous systems, but they do make use of the potential difference across membranes for communication.

The energy and materials that wheat and barley cereals need for growth from the time of germination until they can carry out photosynthesis, are stored in the endosperm (Figure 15.4). Four enzymes break down the amylose and amylopectin in endosperm, but one of them, α-amylase, is not present in the grain and needs to be synthesised during germination (see Figure 15.5).

▲ **Figure 15.4** A vertical section through a wheat grain. The embryo is at the base and the rest is endosperm. The aleurone layer is between the endosperm and the outer coat ×6

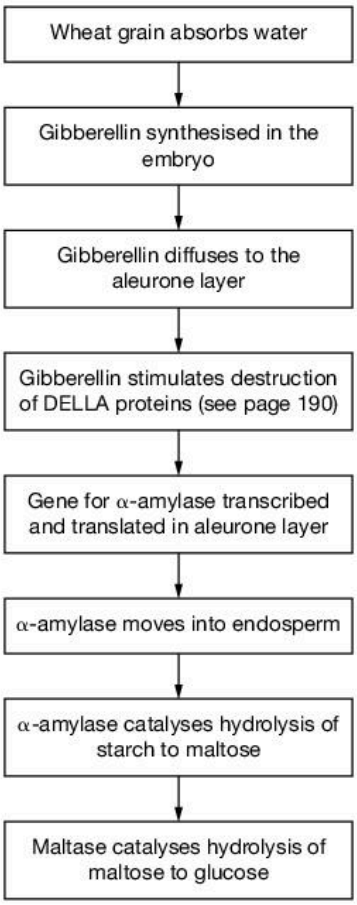
Gibberellins and height

Genetically dwarf varieties of peas, *Pisum sativum*, are homozygous for a gene that codes for an enzyme in the metabolic pathway that produces gibberellin. The stem length gene, ***Le/le***, codes for the last enzyme in the pathway. The dominant allele, ***Le***, codes for a functioning enzyme permitting normal gibberellin synthesis and making the 'tall' phenotype. The mutant allele, ***le***, is recessive so homozygous plants, ***lele***, are genetically dwarf. Gibberellin stimulates the cell wall enzyme (known as XET) that breaks bonds within hemicellulose molecules to loosen the cell wall in a similar way to expansins (see next paragraph).

Auxins stimulate elongation growth

The auxin IAA is synthesised in growing shoot tips and diffuses down the stem. IAA is thought to stimulate the phosphorylation of proton pump proteins in cell surface membranes so increasing their activity. Pumping out protons has the following effects:

- decreases the pH of cell walls – this breaks the bonds between cellulose microfibrils and the surrounding matrix made of hemicelluloses and pectins. It also activates cell wall proteins known as expansins that break hydrogen bonds between cell wall polymers
- changes the potential difference across the membrane stimulating potassium influx channels to open
- potassium ions diffuse into cells so decreasing water potential
- water enters by osmosis through aquaporins increasing turgor pressure
- the increase in turgor pressure causes the cell walls to stretch allowing plant cells to grow in size.



▲ **Figure 15.5** The action of gibberellin in the germination of a wheat grain

The rapid response of the Venus fly trap

The internal surfaces of the leaves of the Venus fly trap have sensory hair cells which are deflected when an insect touches them. Deflection of the hair is a mechanical stimulus that activates calcium ion channel proteins at the base of the hair. Calcium ions enter the cells causing the cell membrane to depolarise. If two hairs are touched within 35 seconds then impulses spread over the leaf along cell surface membranes. The action potentials are similar to those in neurones, but the ion flow is different and they last longer.

When the impulse reaches the hinge region of the trap, pump proteins in the cell surface membranes respond by pumping protons into the cell walls. The decrease in pH in the cell wall loosens the cross links between the cell wall components. Calcium ions enter the cell to decrease the water potential. Water enters through aquaporins and these cells become turgid. The rapid change in turgidity and a change in the tension within cell walls of the hinge cells are thought to be responsible for rapid closure of the trap.

Raise your grade

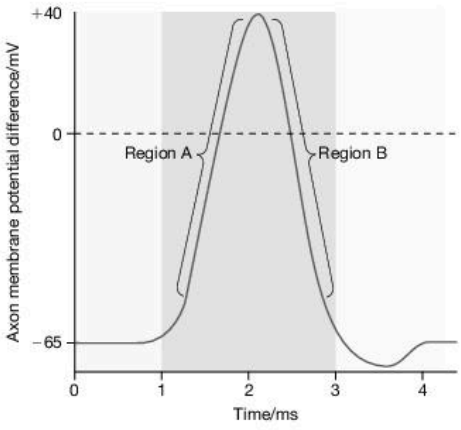
1 (a) Sodium and potassium ions are involved in the transmission of impulses in the nervous system.

State and explain the distribution of these ions when an axon is at resting potential. [5]

The concentration of sodium ions is higher outside the axon than inside. ✔ The concentration of potassium ions is higher inside the axon than outside. ✔ Sodium/potassium pumps in the axon membrane pump three sodium ions out and two potassium ions in for every molecule of ATP used. ✔ Both ions are unable to diffuse through the hydrophobic core of the axon membrane ✔ and, at rest, the voltage-gated channel proteins are closed. ✔ Potassium ions can diffuse out through 'leak' channel proteins, ✔ but they are kept within the axon by negatively-charged molecules, such as proteins. ✔

A very full explanation of a difficult question. The candidate makes good use of ideas from Unit 4.

The figure shows an action potential in the axon of a sensory neurone.



(b) Explain what happens to change the membrane potential from:

(i) −65 mV to +40 mV (region A) [3]

The axon membrane depolarises as voltage-gated sodium ion channel proteins open. ✔ There is a positive feedback so that more and more of these open. ✔ Sodium ions move through the membrane ✔ into the neurone down their electrochemical gradient as there is a high concentration outside and a negative charge inside. ✔

The candidate makes good use of the appropriate terminology.

(ii) +40 mV to −70 mV (region B). [3]

The membrane repolarises. ✔ Voltage-gated sodium ion channel proteins close so that sodium ions cannot diffuse in. ✔ Voltage-gated potassium ion channel proteins open ✔ and potassium ions diffuse out down their concentration gradient to restore the potential difference to resting potential. ✔

A good answer. The candidate explains that the efflux of potassium ions is responsible for the decrease in potential difference in the falling phase of the action potential.

(c) Explain the role of the refractory period in the transmission of impulses. [3]

The refractory period is caused by the closing of sodium ion channel proteins. ✔ No matter how much depolarisation occurs they do not open for a short while. ✔ This ensures that

an action potential is a brief change in potential difference. ✔ The length of the refractory period determines how many action potentials can occur over a certain period of time. So it determines the maximum frequency of impulses. ✔

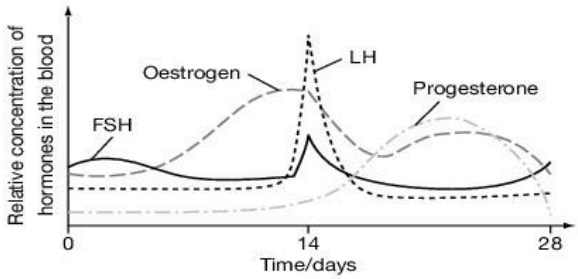
The candidate shows a good understanding of the cause of the refractory period and its role in determining the frequency of impulse transmission.

(d) State how sensory neurones send information about the strengths of stimuli to the CNS. [1]

If there is a weak stimulus, e.g. slight deflection of a Pacinian corpuscle, then the depolarisation of the neurone does not reach the threshold potential so there are no impulses. Above threshold, the frequency of impulses increases as the strength of the stimulus increases. ✔

The first part of the answer is correct, but does not address the question. However, the last sentence is the expected answer so gains one mark.

2 The graph shows changes in concentrations of four hormones in the blood during the menstrual cycle.



(a) State the precise sites of release of each of the hormones shown in the graph. [4]

FSH and LH – pituitary gland. ✔
Oestrogen – ovary. Progesterone – ovary. ✔

These answers are not precise enough for full marks, but two marks are awarded for identifying the correct organs that release these hormones. The first answer should be the *anterior* pituitary gland; in the ovary, oestrogen is released from the follicle and progesterone from the corpus luteum.

(b) State what event occurs at day 14 and use information from the graph to explain your answer. [2]

Ovulation. ✔ The concentrations of FSH and LH increase steeply to a peak just before day 14. ✔

Correct answer. The graph shows the surge in FSH and LH that coincides with ovulation.

(c) Describe the role of progesterone during the menstrual cycle. [3]

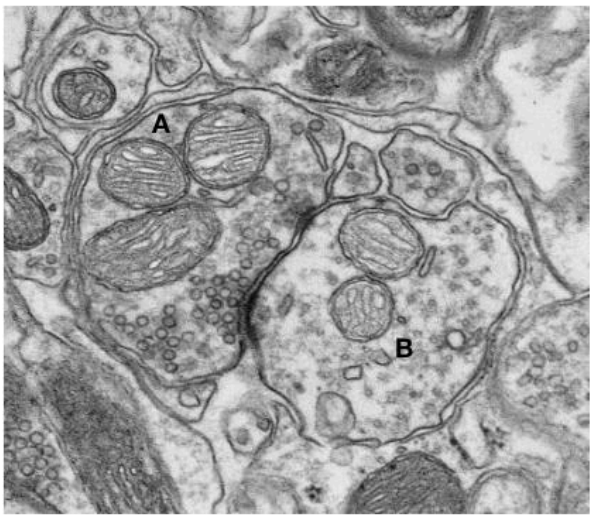
Progesterone is secreted in the second half of the menstrual cycle. It maintains the thickness of the uterine lining ✔ for implantation of an embryo if fertilisation has occurred. ✔ It also inhibits the release of FSH from the anterior pituitary gland so that another cycle does not start. ✔

Correct answer.

Exam-style questions

Structured questions (Paper 4)

1 The electron micrograph shows a section through the junction between two neurones, **A** and **B**, in the spinal cord.



× 30 000

(a) (i) The junction between the two neurones is a cholinergic synapse. State what this means. [1]

(ii) With reference to the electron micrograph, explain how the structure of a synapse ensures that impulses travel in one direction. [3]

(b) Stimulation of a relay neurone by a sensory neurone does not always result in impulse transmission.

Explain why a relay neurone may not respond to stimulation by a sensory neurone. [3]

(c) The speed of nerve impulses from touch receptors in the skin is about 80 m s^{-1}, but from some pain receptors it is about 1.0 m s^{-1}.

Suggest and explain what causes the difference in speed of impulses from these receptors. [3]

2 The leaves of the Venus fly trap catch and digest insects. The trap is a specialised leaf with two lobes either side of a central midrib, which acts as a hinge. The lobes are red inside and have sensitive hairs that respond when touched so causing the lobes to shut.

The sensitive hairs generate a receptor potential when they are stimulated. The trap only shuts if at least two hairs are stimulated within 35 s of each other. Once stimulated the trap shuts partially within one second. After a short while the trap closes tightly.

(a) Explain how the receptor potential is produced. [2]

(b) (i) Suggest the advantage of requiring two deflections of different hairs before closure of the trap. [1]

(ii) Suggest why the trap does not close tightly at first. [1]

(c) The Venus fly trap does not have specialised cells like neurones to conduct impulses. Explain how impulses travel across the leaf to the midrib. [3]

(d) Venus fly trap plants grow in nutrient-poor, waterlogged soils in the Carolinas in the USA.

Suggest how trapping insects benefits these plants in this habitat. [2]

Free response questions (Paper 4)

3 (a) The following are found at synapses:

voltage-gated calcium ion channel proteins, vesicles, mitochondria, chemical-gated sodium ion channel proteins, acetylcholinesterase

Describe the function of these structures in the transmission of an impulse across a synapse. [10]

(b) Outline the steps that occur following the transmission of an impulse across a neuromuscular junction that result in contraction of a muscle fibre. (Do not include the mechanism of muscle contraction in your answer.) [5]

4 (a) Compare the roles of the nervous system and the endocrine system in maintaining a homeostatic equilibrium in mammals when the external temperature becomes very cold and when the water potential of the blood decreases. [10]

(b) Outline the roles of auxins in controlling elongation growth in plants. [5]

extra questions available online

16 Inherited change

Key points

- ☐ Explain what is meant by homologous pairs of chromosomes.
- ☐ Explain the meanings of the terms haploid and diploid and the need for a reduction division (meiosis) prior to fertilisation in sexual reproduction.
- ☐ Describe the behaviour of chromosomes in plant and animal cells during meiosis, and the associated behaviour of the nuclear envelope, cell surface membrane and the spindle.
- ☐ Explain how crossing over and random assortment of homologous chromosomes during meiosis and random fusion of gametes at fertilisation lead to genetic variation including the expression of rare, recessive alleles.
- ☐ Outline the role of meiosis in gametogenesis in humans and in the formation of pollen grains and embryo sacs in flowering plants.
- ☐ Explain the terms gene, locus, allele, dominant, recessive, codominant, linkage, test cross, F1 and F2, phenotype, genotype, homozygous and heterozygous.
- ☐ Use genetic diagrams to solve problems involving monohybrid and dihybrid crosses (to include autosomal linkage, sex linkage, codominance, multiple alleles, gene interactions) and test crosses.
- ☐ Use the chi-squared test to test the significance of differences between observed and expected results.
- ☐ Explain that gene mutation occurs by substitution, deletion and insertion of base pairs in DNA and outline how such mutations may affect the phenotype.
- ☐ Explain the relationship between genes, enzymes and phenotype with respect to the gene for tyrosinase involved in melanin production.
- ☐ Outline the effects of mutant alleles on the phenotype in albinism, sickle cell anaemia, haemophilia and Huntington's disease.
- ☐ Distinguish between structural and regulatory genes and between repressible and inducible enzymes.
- ☐ Explain genetic control of protein production in a prokaryote using the *lac* operon.
- ☐ Explain the function of transcription factors in gene expression in eukaryotes.
- ☐ Explain how gibberellin activates genes by causing the breakdown of DELLA protein repressors, which normally inhibit factors that promote transcription.

Chromosomes become separate structures during nuclear division after they have replicated. The chromosomes are double structures consisting of two molecules of DNA that are packaged tightly to make two sister chromatids. As replication is precise, the two sister chromatids usually have identical sequences of base pairs and therefore are genetically identical.

Figure 16.1 shows the chromosomes of a species of deer at metaphase of mitosis. There are three pairs of **homologous chromosomes** (2n = 6): two pairs are **autosomes** and one pair are the **sex chromosomes** (XX).

The two chromosomes that form an homologous pair have the same genes in exactly the same positions along the DNA. The genes on the two chromosomes may or may not have identical sequences of base pairs. If the sequences of base pairs are not identical for a particular gene, then the chromosomes have different alleles of that gene. This is often the case in many species.

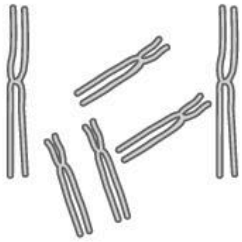
Key terms

Homologous chromosomes: a pair of chromosomes that have the same shape, same position of the centromere and have the same genes in the same sequence. The alleles of the genes are often different on the two chromosomes.

A Level

Sets of chromosomes

Human cells have a **diploid** number of 46. One set of 23 chromosomes is inherited from the mother and the other set from the father. Each set contains one example of each type of chromosome. Females having 22 plus an X chromosome and human males having 22 plus an X or a Y chromosome. At **meiosis** the chromosome number is halved to form **haploid** nuclei.

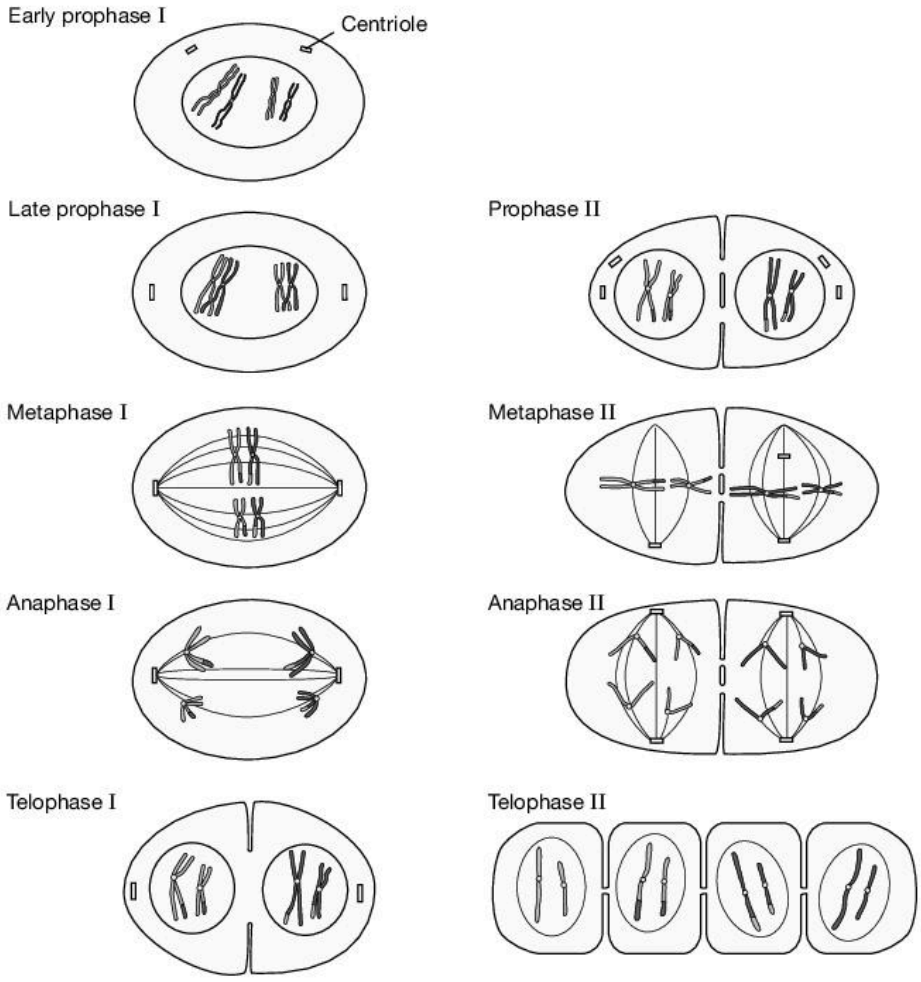


▲ **Figure 16.1** Chromosomes of a female Indian muntjac deer, *Muntiacus muntjak*, at metaphase of mitosis

Meiosis

Meiosis is important in life cycles as it generates variation in the nuclei that are produced. Meiosis is carefully controlled so that each daughter cell gains one complete set of chromosomes with a random mixture of maternal chromosomes (from the egg nucleus) and paternal chromosomes (from the sperm nucleus). At fertilisation, fusion of male and female gamete nuclei ensures that the diploid number is restored.

In meiosis, the nucleus goes through two divisions: homologous chromosomes separate in **meiosis I** and chromatids separate in **meiosis II.**



▲ **Figure 16.2** The stages of meiosis in an animal cell to produce spermatozoa. In prophase of meiosis I homologous chromosomes pair to form bivalents and form chiasmata

Key terms

Autosomes: all chromosomes *except* the sex chromosomes.

Sex chromosomes: the pair of chromosomes that determine sex, known as X and Y. The homogametic sex is XX; the heterogametic sex is XY. In mammals, the homogametic sex (XX) is female.

Diploid: a cell, nucleus or an organism having two sets of chromosomes (2n).

Meiosis: nuclear division in which homologous chromosomes pair to form bivalents. The chromosome number is halved during meiosis I so the parent diploid nucleus gives rise to four haploid nuclei that are genetically different to one another and to the parent nucleus. There are two divisions – meiosis I followed by meiosis II.

Haploid: a cell, nucleus or an organism having one set of unpaired chromosomes (n) (or in polyploid organisms having half the number of sets as body cells).

★ Exam tip

Random assortment during meiosis generates 2^n different combinations of maternal and paternal chromosomes, e.g. in humans this is 2^{23} (= 8 388 608). At fertilisation the number of possible combinations is therefore $2^{23} \times 2^{23}$ (= 2^{46}) in a human zygote.

★ Exam tip

Make sure that you always spell **mitosis** and **meiosis** correctly. A common misspelling of meiosis looks like mitosis.

Meiosis promotes variation

Figure 16.3 and 16.4 show that **random assortment** and **crossing over** are sources of genetic variation. All haploid gametes produced are genetically different.

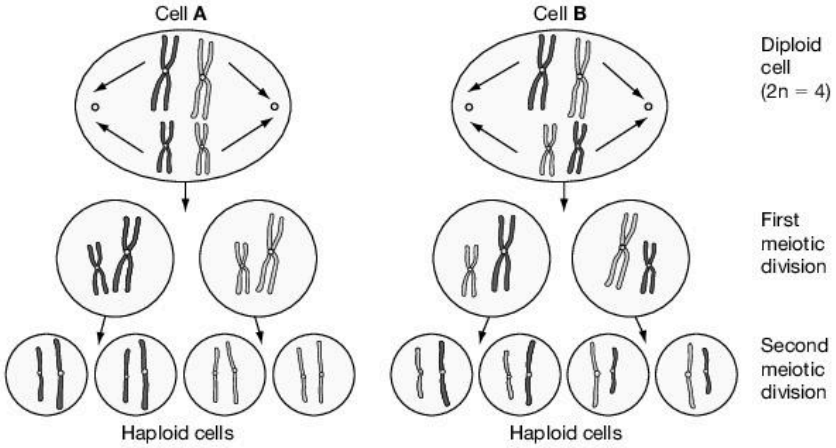
Random assortment

The alignment of bivalents at metaphase I is entirely random. The chromosomes can either line up as shown in cell A or as in cell B in Figure 16.3. There is a 50% chance of alignment A and a 50% chance for B.

Crossing over

The breakage and exchange of DNA between non-sister chromatids of homologous pairs that occurs early in prophase I is crossing over.

As a result, a chromosome has one part that is maternal in origin and another that is paternal. This gives different combinations of alleles of different genes. Nucleotides are not gained or lost and each gene remains in the same position (locus) on the chromosome.



▲ **Figure 16.3** Random assortment of homologous chromosomes in meiosis I. This is one cause of inherited variation since each nucleus contains a mixture of maternal chromosomes (shaded) and paternal chromosomes (unshaded)

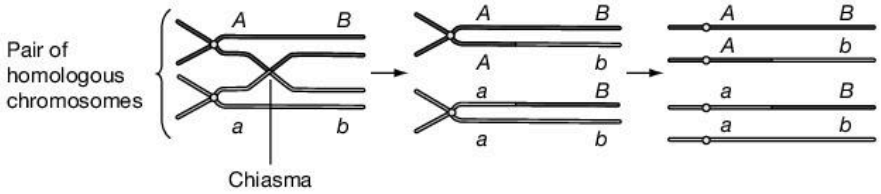


Figure 16.4 Crossing over between chromatids of a homologous pair and the effect on the alleles of two genes

Genetic differences between individuals are caused by the different alleles (variants of the genes) they have inherited. The term **recombination** applies to the new combinations of alleles that have occurred as a result of meiosis. Further variation is introduced at fertilisation since males and females usually have different genotypes and show random mating. There is also random fusion of gametes at fertilisation which, with meiosis, increases the chances that rare, recessive alleles are expressed in homozygous organisms. The phenotype that results is exposed to selection. Most of the genetic diseases described in Table 16.3 are caused by rare, recessive alleles.

Gametogenesis

Gametogenesis in humans, as in all animals, involves the division of nuclei by meiosis and cytoplasmic division to produce the ovum or spermatozoa.

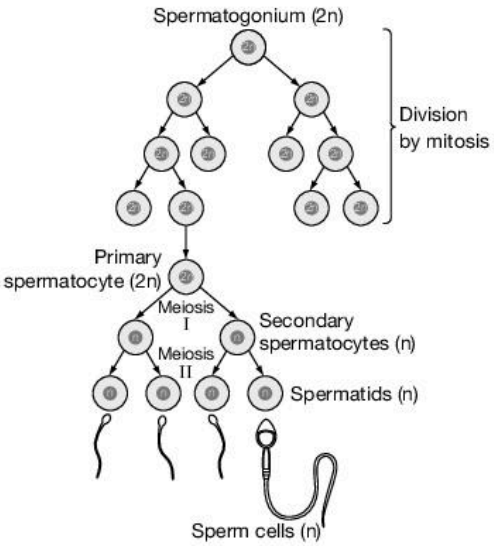
Spermatogenesis, which begins at puberty, is a continuous process for the production of spermatozoa that occurs in seminiferous tubules inside the testes (Figure 16.5). **Oogenesis** is the production of an ovum and occurs in the follicles of the ovary. It is an interrupted process that begins before birth (Figure 16.6). The primary oogonia form primary oocytes which remain in prophase I at birth. During each menstrual cycle, a primary oocyte completes meiosis I. The secondary oocyte formed is at metaphase II at ovulation and completes meiosis II at fertilisation to produce the ovum.

Key term

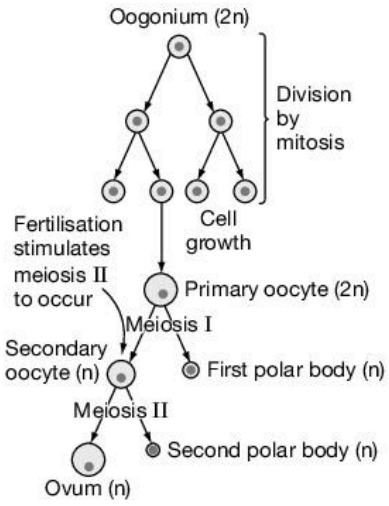
Random assortment: the random arrangement of the alleles of two or more genes found on separate chromosomes as a result of the random arrangement of homologous pairs on the spindle equator in metaphase I.

Crossing over: the breakage and exchange of parts of **non-sister chromatids** of homologous chromosomes during meiosis I.

Recombination: the new combinations of the alleles of two or more genes as a result of (1) random assortment of genes or (2) the result of crossing over of genes. These are the major causes of genetic variation from generation to generation.



▲ **Figure 16.5** Spermatogenesis



▲ **Figure 16.6** Oogenesis

Note in Figure 16.5 the equal division of the cytoplasm to give four haploid sperm cells. One spermatozoan is enlarged to show its structure.

Note in Figure 16.6 the unequal division of the cytoplasm to give three haploid nuclei, but one large ovum and two tiny polar bodies.

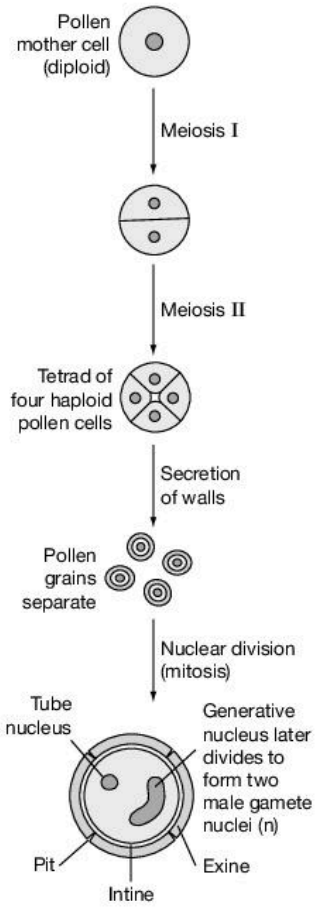
Link

Unit 17 explores the advantages of variation in terms of selection and adaptation to the environment.

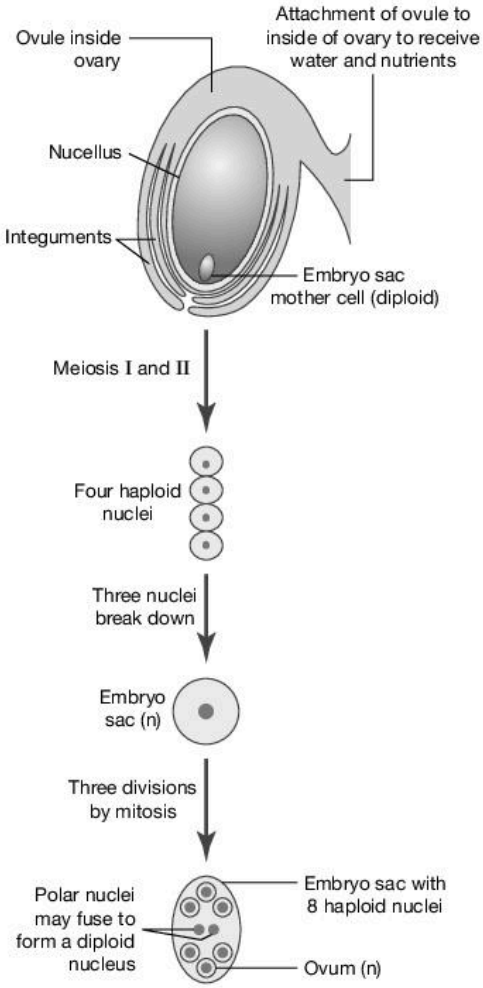
Flowers are made for sexual reproduction. Most flowers are hermaphrodite as they have both male and female structures in which cells divide by meiosis to form spores. The male spore is the pollen grain that gives rise to the male gamete; the female spore is a multinucleate structure that gives rise to the female gamete (ovum) and, unlike the pollen grain, is retained within the flower.

Link

Mitosis is a separate process in gametogenesis in animals to form many spermatogonia and oogonia for the process of meiosis.



▲ **Figure 16.7** Pollen grain production in a pollen sac of an anther



▲ **Figure 16.8** The development of the embryo sac in the carpel

Exam tip

Follow the production of pollen grains in the diagram. Make sure you look at prepared slides or photomicrographs of pollen sacs. If you look at sections of anthers at different stages of development, you can see cells in the stages of meiosis.

The embryo sac is the female spore – the female equivalent of the pollen grain.

Terminology in genetics

You must be able to define and use the following terms:

Gene A length of DNA (sequence of nucleotides) that codes for a single polypeptide. In genetic diagrams, letters are used to designate structural genes, e.g. **A/a**, **B/b**.

Locus (plural: loci) The position of a gene on a chromosome.

Allele An alternative form (or variant) of a gene. There may be two alleles of a gene (e.g. **A** and **a**). Some genes have multiple alleles, e.g. the ABO blood group gene which has three (I^A, I^B and I^o).

Genotype The genetic constitution of an organism, restricted to the alleles of the genes being considered, for example **AA**, **Aa** and **aa** or **AABB**, **AaBb**, etc.

Phenotype The features of an individual which are the result of gene expression and interaction between the genotype and the environment. The phenotype includes outward appearance (morphology), anatomy, physiology, histology, cytology and biochemistry.

Dominant allele has the same effect on the phenotype when homozygous (e.g. **AA**) as when heterozygous (e.g. **Aa**). Upper-case letters are used for dominant alleles.

Recessive allele only has an effect on the phenotype when homozygous (i.e. **aa**). Lower-case letters are used for recessive alleles, and are the *same* letter as used for the dominant allele.

Homozygote A genotype in which the two alleles of a gene are identical, e.g. **AA** and **aa**. Individuals with genotypes like these are described as homozygous.

Heterozygote A genotype in which the two alleles of a gene are different, e.g. **Aa**. Individuals with genotypes like this are heterozygous.

Codominant alleles Alleles that are both expressed in the phenotype of a heterozygote. A heterozygote has a phenotype that differs from the phenotypes of the two homozygotes (e.g. pink flowers as distinct from red and white). An upper-case letter is used for the gene and the alleles are shown by superscripts, e.g. I^A and I^B for the two codominant alleles of the ABO blood group gene.

Parental generation In a genetic diagram, these are the first individuals to be crossed.

F1 generation The first generation resulting from crossing two *homozygotes* in the parental generation. If the parents are not homozygous the term 'offspring 1' is used.

F2 generation The second generation resulting from crossing individuals of the F1 generation. If the individuals in the parental generation were not homozygous, then the term 'offspring 2' is used.

Sex linkage Genes that occur on the sex chromosomes are sex linked as their inheritance is associated with the determination of sex by the X and Y chromosomes. In mammals, the Y chromosome has few genes so sex linkage usually refers to gene loci on the X chromosome. The alleles are often shown as superscripts following the sex chromosome, e.g. X^AX^A, X^AX^a and X^AY. The male only has one allele.

Autosomal linkage / Linkage Two or more genes that occur together on an autosome (chromosome other than a sex chromosome).

★ Exam tip

For ABO blood group alleles, do *not* use **A** and **B** as that suggests they are different *genes* rather than different *alleles*.

★ Exam tip

Make sure you know how to use all these terms, particularly *gene* and *allele*. These are often used incorrectly.

Genetic diagrams

Genetic diagrams are used to show how the genes for different features are inherited and should always be set out clearly so that examiners can follow your reasoning.

▼ **Table 16.1** The rules for making genetic diagrams

Step 1	Describe the gene or genes concerned	State the feature or features controlled by the gene or genes.
Step 2	Identify the allelic features	Two alleles or more for each gene. Use information provided to work out if alleles show dominance and recessiveness or if codominance is shown
Step 3	Choose symbols for the alleles	Capital letter for dominant allele; lowercase letter for the recessive allele. Use the *same* letter for both. For codominant alleles or multiple alleles, use a capital letter for the gene and superscripts for the alleles (see page 188).
Step 4	Give the phenotype and genotype of the parents	Genotype has two alleles for each gene, except in sex-linkage, where a male will only have one allele. When writing a genetic diagram that shows sex linkage include the X and Y chromosomes in the genotypes (see the answer to Question 2(b) on page 191).
Step 5	State the genotype of the gametes	Always write genotypes of gametes inside circles: monohybrid crosses will have one letter in each circle; dihybrid crosses will have two. Write down the different types of gamete. If they are all the same just write down one genotype in a circle. When writing a genetic diagram that shows sex linkage (see page 188) then include the X and Y chromosomes in the genotypes.
Step 6	Show all the possible genotypes that will result from fusion of gametes at fertilisation	Use a Punnett square to show all the possible fusions whenever there are gametes with two or more different genotypes. The Punnett square gives the probability of each offspring genotype, not the actual numbers of offspring.
Step 7	Write out the different genotypes and give the phenotype of each one	See how this is done in the genetic diagrams on the following pages and in the Mark schemes for the questions at the end of this Unit.
Step 8	Write out the probability of each phenotype and express the answer as a ratio	You may be given the numbers of offspring. Note that these are numbers for different categories (categorical data). Always divide the numbers by the smallest number to find the overall phenotypic ratio, e.g. 66 : 33 is 2 : 1.

★ Exam tip

Make sure the difference between lower case and upper case letters for allele symbols are clear when the letters are similar, e.g. Pp, Cc.

Monohybrid cross

Example involving dominance and recessiveness

The genetic diagram opposite shows a monohybrid cross involving the inheritance of one gene – the gene for wing length in *Drosophila melanogaster*. There are two alleles: long wing (also known as wild type) (**W**) and vestigial wing (**w**). Vestigial means very small.

During meiosis in the F1 flies the two alleles (**W** and **w**) separate because they are on homologous chromosomes that separate during meiosis I. This separation is **segregation of a pair of alleles**.

★ Exam tip

A monohybrid cross reveals the inheritance pattern of a single gene with two or more alleles.

★ Exam tip

The terms F1 and F2 are correct here as they are being used in a cross in which the parental individuals are homozygous.

parental phenotypes	male	×	female
parental genotypes	long wing (wild type) **WW**		vestigial wing **ww**
parental gametes	(W)	+	(w)
F_1 *genotype*	**Ww**		
F_1 *phenotype*	all long wing (wild type)		
F_1 *phenotypes*	long wing	×	long wing
F_1 *genotypes*	**Ww**		**Ww**
F_1 *gametes*	(W), (w)	+	(W), (w)

		male gametes (W)	male gametes (w)
female gametes	(W)	**WW**	**Ww**
female gametes	(w)	**Ww**	**ww**

F_2 genotypes and phenotypes

F_2 phenotypic ratio

WW long wing : **2Ww** long wing : **ww** vestigial wing

3 long wing : 1 vestigial wing

If the cross is carried out using females with long wings and males with short wings (the reciprocal cross), the same results are obtained. This shows that the gene for wing length is not linked with the inheritance of the chromosomes that determine sex and explains why sex chromosomes are *not* included in the genetic diagram.

All individuals with the recessive characteristic are homozygous recessive. However, the genotype of an individual with the dominant characteristic could be homozygous dominant or heterozygous. We can find out by doing a **test cross**. This involves crossing the individual with unknown genotype with an individual that is homozygous recessive.

Key term

Test cross: a cross designed to discover the genotype of an organism by studying the phenotypes of the offspring. The individual with the unknown genotype is mated with another that is homozygous recessive for the gene or genes concerned.

★ Exam tip

Write a genetic diagram to explain the answer given in this Worked example. Then check your diagram with the one provided on the support website.

Worked example

A student used test crosses to identify the genotypes of some male fruit flies with long wings. In some crosses (**A**) all the offspring had long wings. In other crosses (**B**) 50% of the offspring had long wings and 50% had short wings.

(a) State the genotypes of the parent fruit flies for crosses **A** and **B**.

(b) Use this example to explain the advantage of carrying out a test cross to identify unknown genotypes rather than crossing two long-winged fruit flies together.

Answers

(a) Cross **A**: (males) **WW** and (females) **ww**. Cross **B:** (males) **Ww** and (females) **ww**.

(b) The offspring of a test cross always inherit a recessive allele (e.g. **w**) from the parent that is homozygous recessive. This means that the allele that the offspring inherit from the parent with the unknown genotype will always be expressed. If it is recessive, then the offspring will show the recessive phenotype (e.g. short wings); if dominant, then the offspring will show the dominant phenotype (e.g. long wings). If the female in this cross was long-winged, then she could be homozygous dominant and all the offspring would be long-winged and that would not tell us anything about the genotype of the male.

Example of monohybrid inheritance involving codominance

The four o'clock plant (Marvel of Peru), *Mirabilis jalapa*, has three different flower colours. If pure bred red-flowered plants are crossed with pure bred white-flowered plants then the F1 plants have pink flowers. They are different from either of the two parental phenotypes. However, if these pink-flowered plants are interbred then the next generation gives all three colours in a 1:2:1 ratio. This F2 phenotypic ratio is the same as the *genotypic* ratio in the previous monohybrid cross. Draw a genetic diagram to confirm this.

★ Exam tip

'Pure bred' (pure bred lines) means that plants or animals showing a particular feature have been bred for several generations and the individuals are known to be homozygous for the gene concerned.

The reason for the intermediate colour (pink) is that both alleles contribute to the phenotype of the heterozygous plants. They are known as **codominant alleles**. The alleles are written as follows: C^R = red and C^W = white, where **C** represents the gene for flower colour and C^R and C^W represents the alleles. This indicates that the alleles are neither dominant nor recessive.

Sex linkage

Sex linkage is the occurrence of a gene on a sex chromosome, usually the X chromosome. Note the following features of sex linkage:

- the recessive phenotype is more common in males than in females
- males cannot inherit the recessive condition from their male parent, but females can
- females can be heterozygous and therefore carriers of the condition, males can never be carriers
- males inherit their Y chromosome from their father and this does not have the loci that are found on the X chromosome
- reciprocal crosses do not give the same offspring phenotypic ratios.

Multiple alleles

Many genes have more than two alleles. There are three alleles at the human ABO blood group locus. The gene symbol is **I** and there are three alleles, I^A, I^B and I^o. I^A and I^B are codominant and both are dominant to I^o. There are six genotypes and four phenotypes as shown in Table 16.2.

Dihybrid cross

A dihybrid cross involves the inheritance of two genes. Wild type fruit flies have long wings and grey bodies. Some have short wings and black bodies. Pure bred flies of both types were crossed and all the offspring had the wild type phenotype. When these F1 flies were crossed all combinations of features appeared in the F2 offspring to give: wild type (long wings, grey body) 650, long wings and black body 198, short wings and grey body 225, short wings and black body 68; an approximate ratio of 9:3:3:1. To get the phenotypic ratio, divide the smallest number into the others. But the ratio of long wings to short wings is about 3:1 and the ratio of grey body to black body is also about 3:1.

The explanation for the ratio is **random assortment** (see page 183). The alleles of the two genes segregate in meiosis independently of one another to produce four genetically different gamete types, because they are on different chromosomes.

Link

If you find random assortment a difficult concept to understand, use models of chromosomes made from modelling clay or pipe cleaners. Attach sticky labels to show the alleles of the two genes and look at Figure 16.3 again more closely. Remember that there is a 50% probability that two pairs of homologous chromosomes will align themselves during metaphase I of meiosis with the dominant alleles of the two genes together.

Interactions between genes

Some features are controlled by two or more genes that interact with each other. The genes involved will show random assortment if they are on different chromosomes, but the ratio obtained in the F2 generation may be different to the 9:3:3:1 of a dihybrid cross where genes do not interact.

Flower colour in blue-eyed Mary, *Collinsia parviflora*, is a good example, as the pigment which gives the petals their colour is produced by reactions that occur in series catalysed by two different enzymes (Figure 16.9).

Exam tip

If a question says that males and females of a certain species were crossed, that does not necessarily mean that the gene involved is sex linked. You need to look for other clues.

▼ **Table 16.2**

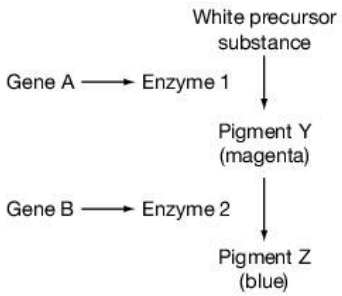
Genotype	Blood group
$I^A I^A$	A
$I^A I^o$	
$I^A I^B$	AB
$I^B I^B$	B
$I^B I^o$	
$I^o I^o$	O

Exam tip

Make a genetic diagram to show how a man who has blood group A and a woman who has blood group B can have children with all four blood groups. Check your diagram with the one provided on the support website.

Exam tip

Make a genetic diagram to explain the results for the F1 and the F2. Check your diagram with the one provided on the support website.



▲ **Figure 16.9** The two enzymes, **1** and **2**, catalyse reactions to produce a flower colour pigment. Gene **A/a** codes for enzyme **1** and gene **B/b** codes for enzyme **2**

Gene **A**/**a** codes for enzyme 1 and gene **B**/**b** codes for enzyme 2. The flowers will be white if enzyme 1 is absent or non-functioning as in plants with genotypes aabb, **aaBB** and **aaBb**. The flowers will be magenta when enzyme 1 is present but enzyme 2 is absent or non-functioning, as in the genotypes **AAbb** and **Aabb**. If the genotype of the plant has dominant alleles of both genes, then the flowers are blue.

Gene mutation

In biology, **mutation** is used to refer to changes in DNA to a gene (**gene mutation**) to a or to a chromosome (chromosome mutation). Changes to individual genes involve changing the sequence of nucleotide bases during replication.

DNA polymerase has a proof-reading capacity and removes and replaces a nucleotide that is in the wrong place. However, this proof reading ability is not 100% accurate, and errors occur during replication.

Substitution. The change of one base pair for another.

Frameshift. The sequence of triplets in DNA changes by the **insertion** of one or more bases or the **deletion** of one or more bases. Changes that are not multiples of three bases will change the entire sequence of amino acids of the polypeptide formed, from the point of mutation onwards.

Neutral mutations are those that have no effect on the amino acid sequence and those that change the sequence, but have no effect on the function of the protein.

DNA to protein to phenotype

Proteins have direct and indirect effects on the phenotype. We have discovered these effects when there are errors in metabolism. For example, if a mutation occurs in the *TYR* gene, either no tyrosinase is produced or a non-functional form is made. Tyrosine is not converted to melanin, so skin pigment (melanin) is not produced, leading to the condition known as albinism in people who are homozygous for the mutation. Table 16.3 lists some human genes, the polypeptides that they code for and the consequences on the phenotype if they are faulty.

▼ **Table 16.3** Four human genes, their most common mutations and the consequences

Gene	Polypeptide affected	Consequences of faulty gene (inheritance pattern)
HBB	β-globin (one of the polypeptides in haemoglobin – see Unit 2 page 19)	**sickle cell anaemia:** many effects, including sickle cell crises when blood cells get stuck in small blood vessels (autosomal recessive)
TYR	enzyme: tyrosinase (transmembrane protein in melanin-producing cells)	**albinism:** no melanin in the skin or hair (autosomal recessive)
HTT	production of abnormal protein that is cut into sections that bind together and accumulate in neurones	**Huntington's disease:** progressive degeneration of brain tissue (autosomal dominant)
F8	blood clotting factor 8 (also called factor VIII)	**haemophilia:** slow clotting time (sex-linked recessive)

★ Exam tip

Make a genetic diagram to show the expected phenotypic ratio when plants heterozygous for both genes, **A** and **B**, are crossed. Check your diagram with the one provided on the support website.

Key term

Gene mutation: a change to the nucleotide sequence of a gene giving a mutant allele of that gene, also known as a gene variant.

★ Exam tip

Frameshifts are likely to have a much greater effect on the protein formed than base substitution. However, a substitution mutation can have profound effects if it changes an amino acid in an important part of a protein (e.g. an active site of an enzyme).

Remember

Look back to the details of the genetic code in Unit 6 page 66 to help you to understand the effects of mutation.

Gene control

Gene control in prokaryotes

Figure 16.10 shows genetic control of protein production in the *lac* operon of the bacterium *Escherichia coli*. The operon consists of a **promoter region** where RNA polymerase binds to start transcription of genes *z*, *y* and *a* to produce a single messenger RNA molecule; an **operator region** where repressor protein binds; and three structural genes – *z* coding for β galactosidase, and *y* and *a* that code for two other proteins needed to metabolise lactose.

In addition to the *lac* operon, there is a regulatory gene somewhere else on the bacterial chromosome that codes for a repressor protein that binds to the operator region inhibiting transcription of the three *lac* genes *z*, *y* and *a*.

β galactosidase is an example of an inducible enzyme as the presence of lactose leads to the transcription of genes in the *lac* operon. The enzyme is not synthesised all the time. Other gene control mechanisms in prokaryotes are involved in the production of repressible enzymes (see Key terms and Question 3 on page 192).

Gene control in eukaryotes

Most genes in eukaryotes have much more complex methods for controlling transcription that involve many proteins that act as transcription factors, either promoting transcription or preventing it.

A single transcription factor cannot start transcription, many such factors are needed. Once the first factor binds to a DNA promoter sequence, secondary factors bind in a predetermined order. Many do not bind to DNA, but bind to other factors instead to form a transcription complex. Once this complex is created, RNA polymerase can bind to DNA and start transcription.

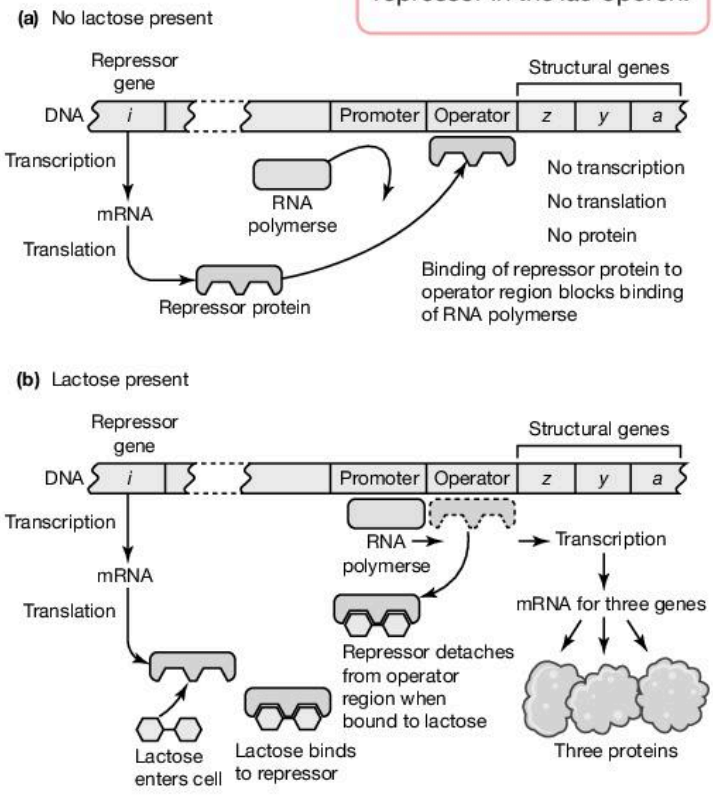
DELLA proteins bind to transcription factors, preventing them from initiating the expression of genes associated with proteins involved in the control of growth in plants. The hormone gibberellin stimulates the destruction of these transcription blocking factors. Once the DELLA proteins are destroyed, activators can stimulate transcription of the genes involved in cell division and cell growth.

Key terms

Operon: a group of genes that are controlled and transcribed together.

Structural gene: a gene that codes for a polypeptide that is not involved in regulating the expression of other genes. The gene that codes for β-galactosidase in the *lac* operon is a structural gene.

Regulatory gene: a gene that codes for a polypeptide (or RNA) that controls the expression of other genes, e.g. the gene that codes for the repressor in the *lac* operon.



▲ **Figure 16.10** How the *lac* operon functions (a) when lactose is absent, and (b) when lactose is present

Key terms

Inducible enzyme: an enzyme that is synthesised only when it is required.

Repressible enzyme: an enzyme that is produced continuously unless its synthesis is inhibited (repressed).

Transcription factor: a protein in a eukaryote that enables RNA polymerase to bind to DNA and initiate (begin) transcription or prevent transcription (switching genes on or off).

Exam tip

It is a good idea to follow an animation as you read about the *lac* operon. Search for 'lac operon animation'.

Link

The roles of gibberellin are described in Unit 15 – see page 177.

Raise your grade

1 A student carried out a genetic investigation with fruit flies, *Drosophila melanogaster*. Two characteristics were observed, body colour and wing shape. The dominant features were grey body and normal wings. The student carried out a test cross on fruit flies that were heterozygous for both gene loci. The results were as follows: grey body and normal wing 83; black body and normal wing 88; grey body and bent wing 78; black body and bent wing 74.

(a) Outline how the student would carry out a test cross. [1]

Fruit flies that show the dominant phenotype (grey body and normal wings) were crossed with flies that were homozygous recessive for both genes (black body and bent wings). ✔

Correct. Test crosses always involve organisms that are homozygous recessive for the gene(s) being investigated.

(b) The student used the chi-squared test to analyse the results. The value of χ^2 is 1.37. Use the table below to make a conclusion from this result. [4]

Degrees of freedom	Probability, *p*				
	0.10	0.05	0.02	0.01	0.001
1	2.71	3.84	5.41	6.64	10.83
2	4.61	5.99	7.82	9.21	13.82
3	6.25	7.82	9.84	11.35	16.27
4	7.78	9.49	11.67	13.28	18.47

There are four categories so the df = 3. ✔ The critical value at $p = 0.05$ is 7.82. The χ^2 value of 1.37 is lower than this so the probability of this result by chance is much more than 0.05 (5%) so the null hypothesis is accepted. ✔ There is no significant difference between the observed and the expected results. ✔ The genes are on different chromosomes and are not linked. The results show that independent assortment has occurred in the formation of gametes in the heterozygous fruit flies. ✔

This is a thoroughly written answer. We do not know for sure that the genes are on different chromosomes, but the results support that hypothesis.

2 A red-eyed male fruit fly was crossed with a white-eyed female fruit fly. The offspring were a mixture of both red-eyed females and white-eyed males in a ratio of 1:1.

(a) Use this example to explain the term *sex linkage*. [3]

Sex linkage is the occurrence of a gene on a sex chromosome, usually the X chromosome ✔. The gene for eye colour is on the X chromosome ✔ so males only have one copy (as they are XY) and always express the allele that they have. ✔

A good answer that uses correct terminology.

(b) The offspring were crossed together. Give the genotypes of the offspring and the phenotypic ratio. [2]

X^RY (red-eyed males); X^RX^r (red-eyed females); X^rY (white-eyed males); X^rX^r (white-eyed females) ✔. The phenotypic ratio is 1:1:1:1. ✔

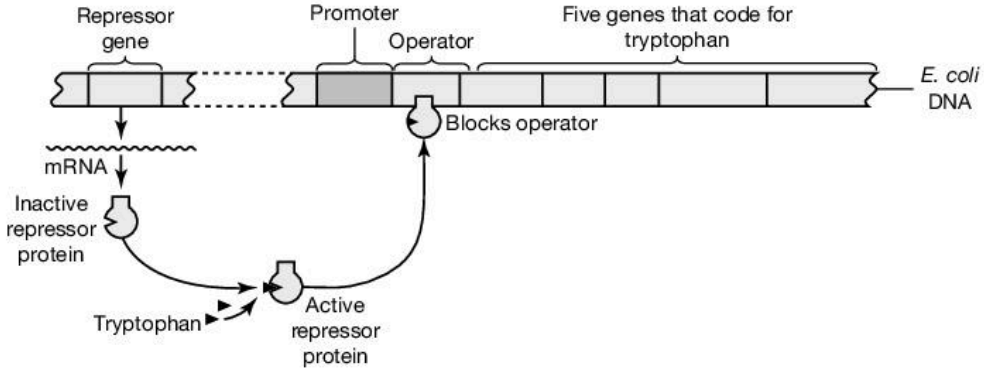
Correct, the offspring of this cross include all four possible phenotypes.

(c) Explain why male fruit flies cannot inherit their eye colour from their fathers. [3]

A male inherits a Y chromosome from his father ✔ and the gene for eye colour is on the X, not the Y ✔. His X chromosome is inherited from his mother.✔

A correct answer.

3 In the bacterium, *Escherichia coli*, there is a short metabolic pathway involving three enzymes that synthesise the amino acid tryptophan. The bacterium only makes tryptophan when there is none available in its surroundings. The five genes that code for the enzymes required for tryptophan synthesis are part of the *trp* operon shown in the diagram.



(a) State **two** advantages of regulating the production of enzymes. [2]

There is no need to produce enzymes if there is nothing for them to do. If tryptophan can be absorbed from the surroundings, *E. coli* does not need to make it as well. This saves energy in protein synthesis. ✔ It also saves using amino acids to make an enzyme that is not needed. ✔

A well-worded answer with good use of unfamiliar information in the diagram.

(b) (i) State **two** ways in which the *trp* operon shown in the diagram differs from the *lac* operon. [2]

The repressor is inhibited by lactose in the *lac* operon, whereas tryptophan activates the repressor in the *trp* operon. ✔ There are five structural genes in the *trp* operon. In the *lac* operon there are only three. ✔

Be prepared to see unfamiliar examples like the *trp* operon in Paper 4. You are expected to use your knowledge to analyse the information provided. Always study all information, including diagrams, provided in questions like this.

(ii) Explain why the control of the *trp* operon differs from the control of the *lac* operon. [3]

The *lac* operon is activated by lactose because proteins are needed to metabolise it to gain energy. Lactose is a respiratory substrate that *E. coli* breaks down to gain energy.✔ The default state for the *trp* operon is to be 'switched on' unless tryptophan is present. When it is present there is no need to make it. ✔ Tryptophan is used to make proteins, it is not broken down like lactose. ✔

A very thorough answer clearly written in the candidate's own words (e.g.'default state'). This shows good application of the principles learnt in this Unit.

Exam-style questions

Structured questions (Paper 4)

1 In shorthorn cattle there is a gene that controls coat colour. The allele $\mathbf{C^R}$ gives a red colour, the allele $\mathbf{C^W}$ gives white. Cattle that are heterozygous for this gene have a coat that is described as roan – a light red colour. Cattle with horns are homozygous for the allele, **p**. Cattle with the dominant allele, **P**, are hornless. Neither of these gene loci is sex linked. The two gene loci are on different chromosomes.

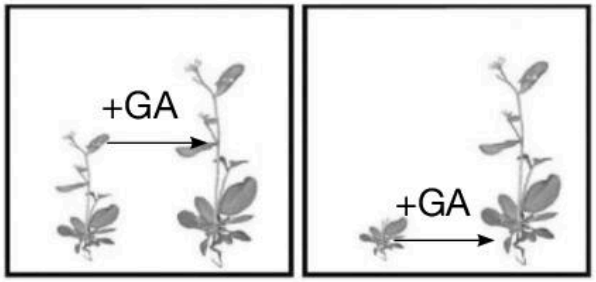
(a) (i) A white cow with horns is mated with a bull that has a red coat and is homozygous for the hornless condition. Draw a genetic diagram to predict what you would expect in the F1 generation. [4]

(ii) Cattle in the F1 generation were mated amongst themselves. Draw a genetic diagram to predict what you would expect in the F2 generation. [6]

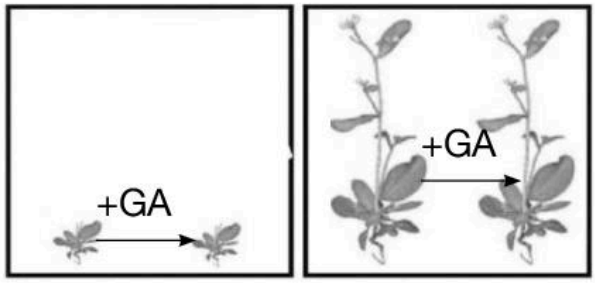
(b) Explain, using this example of shorthorn cattle, the effect of dominance and codominance on phenotypic variation. [4]

2 One of the roles of the plant hormone gibberellin (GA) is to control the growth of stems. There are three types of mutations that have been identified in thale cress, *Arabidopsis thaliana*, that affect the action of gibberellin on growth. Plants with each of these mutations were treated with gibberellin. The plants were grown from seed and were all the same age when they were treated with GA. The drawings show the plants before and after treatment with gibberellin. The drawings were made at the same time after application of GA. The wild type plants do not have any of these mutations.

Group **A** Group **B**



Group **C** Group **D**



(a) (i) Compare the growth of the plants in groups **A** and **B**. [2]

(ii) Suggest an explanation for the response of the plants in group **B** to the supply of gibberellin. [3]

(b) Explain why the plants in group **C** do not respond to the gibberellin treatment in the same way as those in group **B**. [3]

(c) (i) Describe how the growth of plants in group **D** differs from group **A**. [2]

(ii) Suggest an explanation for the growth of plants in group **D**. [3]

Free response questions (Paper 4)

3 **One** role of meiosis in life cycles is to generate genetic variation in the next generation.

(a) Explain how the events that occur during meiosis are responsible for generating genetic variation in the next generation. [10]

(b) Some organisms have life cycles with short diploid stages as meiosis occurs soon after fertilisation. These organisms are haploid for most of their life cycle. One such organism is *Plasmodium* that causes malaria. Discuss the advantages and disadvantages of being haploid for most of the life cycle. [5]

4 (a) Use the example of tyrosinase to explain the link between genes and phenotype. [7]

(b) Sickle cell anaemia is an inherited disease. Explain the genetic cause of sickle cell anaemia and how it is inherited. [8]

extra questions available online

17 Selection and evolution

Key points

- ☐ Describe the differences between continuous and discontinuous variation.
- ☐ Explain the genetic basis of continuous and discontinuous variation.
- ☐ Explain, with examples, how the environment may affect the phenotype of plants and animals.
- ☐ Use the *t*-test to compare the variation of two different populations.
- ☐ State the general theory of evolution that organisms have changed over time.
- ☐ Discuss the molecular evidence that reveals similarities between closely related organisms with reference to mitochondrial DNA and protein sequence data.
- ☐ Explain that natural selection occurs as populations have the capacity to produce many offspring that compete for resources; in the 'struggle for existence' only the individuals that are best adapted survive to breed and pass on their alleles to the next generation.
- ☐ Explain why genetic variation is important in selection.
- ☐ Explain, with examples, how environmental factors can act as stabilising, disruptive and directional forces of natural selection.
- ☐ Use the Hardy–Weinberg principle to calculate allele, genotype and phenotype frequencies in populations and explain situations when this principle does not apply.
- ☐ Explain how selection, the founder effect and genetic drift may affect allele frequencies in populations.
- ☐ Explain how speciation may occur as a result of geographical separation (allopatric speciation), and ecological and behavioural separation (sympatric speciation).
- ☐ Explain the role of pre-zygotic and post-zygotic isolating mechanisms in the evolution of new species.
- ☐ Explain why organisms become extinct, with reference to climate change, competition, habitat loss and killing by humans.
- ☐ Describe how selective breeding (artificial selection) has been used to improve the milk yield of dairy cattle.
- ☐ Outline the following examples of crop improvement by selective breeding:
  - the introduction of disease resistance to varieties of wheat and rice
  - the incorporation of mutant alleles for gibberellin synthesis into dwarf varieties so increasing yield by having a greater proportion of energy put into grain
  - inbreeding and hybridisation to produce vigorous, uniform varieties of maize.

Variation

Types of variation

Variation refers to differences between organisms. These can be phenotypic or genotypic differences. Phenotypic variation is morphological, anatomical, physiological, biochemical and behavioural. Genotypic variation occurs because genes have two or more alleles.

Some phenotypic variation is a direct reflection of genotypic variation, for example, the codominance in flower colour in *Mirabilis jalapa* (see Unit 16 page 187) and the environment plays little or no part.

Link

See the support website for Practical skills on types of data and Unit 16 page 185 on the differences between phenotype and genotype.

We can identify phenotypic variation at two levels:

- **interspecific variation** - the differences *between* different species
- **intraspecific variation** - variation *within* a species – the difference between individuals in the same species.

Intraspecific variation can be:

- **discontinuous:** variation in a feature which has distinct categories without any intermediates – the differences between individuals are qualitative
- **continuous:** variation in a feature that shows a range between two extremes with many intermediates – the differences between individuals are quantitative.

Exam tip

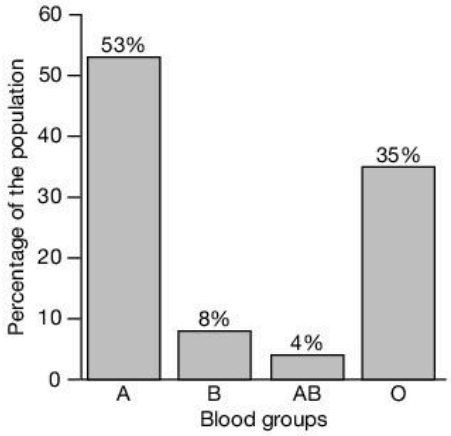
Biologists use interspecific variation to classify different species. Intraspecific variation is used in the understanding of evolution and selection.

Exam tip

Examples of discontinuous variation in humans include albinism and haemophilia (see Table 16.3 on page 189). These have genetic causes.

Discontinuous variation

Where the variation has a genetic cause, it is usually owing to the effect of one or a few genes and the environment has little or no effect.
Data on discontinuous variation is usually presented in the form of bar charts because each category is a separate group (Figure 17.1).



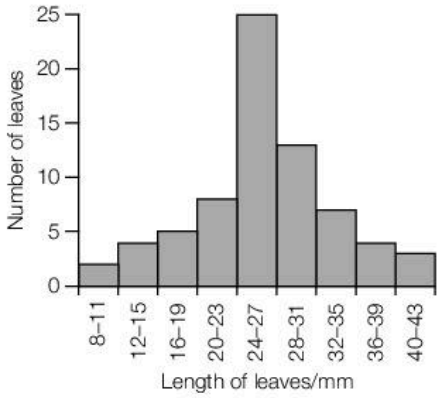
▲ **Figure 17.1** Bar chart to show percentages of the population of Portugal with different ABO blood groups

Continuous variation

Examples of continuous variation include height, length and body mass. The tally table (Table 17.1) and histogram (Figure 17.2) show how data for continuous variation are presented.

▼ **Table 17.1** Tally table for leaf length

Class / mm	Tally	Number	Percentage frequency
8–11	//	2	4
12–15	////	4	8
16–19	###	5	10
20–23	### ///	8	16
24–27	### ###	10	20
28–31	### ### ///	13	26
32–35	### //	7	14
36–39	/	1	2
Total	50	50	100



▲ **Figure 17.2** Histogram to show the variation in a sample of leaves

Continuous variation is determined by genes and by the environment. Together many genes have a polygenic effect as each gene contributes to the phenotypic feature. The genes involved may have multiple alleles at each locus influencing the feature. Also, environmental factors (e.g. for leaves these are light, water and nutrient availability), have the effect of smoothing out the variation to give a **normal distribution** (bell shape) **curve**. You can see this if you draw a line through the centre of the bars on the histogram in Figure 17.2.

Exam tip

The term population will be used often in this chapter. It is all the individuals of one species that live in the same area at the same time.

Link

If you are not sure when and how to draw bar charts and histograms, read Unit 20 Practical assessment page 238.

Effect of the environment on phenotype

Some genes only become effective in certain circumstances. The specific type of tyrosinase in Siamese cats is sensitive to heat and does not function at normal body temperature. It is only active in colder temperatures, such as those at the extremities of the animal . If the face, paws and tail are kept warm during the early life of a kitten, the enzyme does not function and the black markings do not appear (Figure 17.3).

Features that show continuous variation are strongly influenced by environmental factors, such as nutrition. For example, most female honey bees develop into worker bees because they are fed on a diet of pollen and nectar. Young female bees destined to become princesses and then queens are fed royal jelly throughout their time as larvae.

The effects of the environment are not inheritable as changes that have occurred to an organism during its lifetime are not transmitted to the genes in the organism's reproductive organs.

Breeders of livestock animals and crop plants often want to improve growth rates and final yields. To find out how much of the variation is due to genes rather than to the environment, the animals and plants that are genetically identical (or nearly so) are kept in different environments and the variation measured. As the genotypes are the same any variation must be due to the environment.

Exam tip

Researchers interested in the effect of the environment on the overall variation in humans can study monozygotic (identical) twins, particularly twins who have been raised apart. They have exactly the same genotypes, but will have experienced different environments since birth (or shortly afterwards).

Link

There is information about tyrosinase in Unit 16 page 189. Siamese cats have a mutation in the *TYR* gene that causes the heat sensitivity.

▲ **Figure 17.3** A Siamese cat showing the cooler areas of the body where tyrosinase has been active making melanin

Evolution – the big idea

Evidence for evolution

The evidence for **evolution** comes from many areas of biology and geology. You need to know about two lines of evidence from biochemistry:

- mitochondrial DNA
- protein sequencing.

Mitochondrial DNA (mtDNA) is a circular loop of DNA found in mitochondria. Animal mtDNA is about 16.5 kb; plant mtDNA is longer, being between 200 and 2000 kb. mtDNA is useful for studying relationships between different individuals, different populations and different species because:

- mtDNA stays relatively constant for many generations as crossing over does not happen in mitochondria
- mitochondria are inherited in the female line (maternal inheritance) as none of the mitochondria from the male gamete survive inside the egg after fertilisation, so all the descendants of one female have identical mtDNA

Key term

Evolution: the process of genetic change that takes place in populations over successive generations leading to the formation of new species.

kb is the abbreviation for kilobase – 1000 base pairs. Human mtDNA is 16 569 bases long and codes for 13 polypeptides as well as the tRNA and rRNA molecules needed for protein synthesis within mitochondria.

- mutations when they happen are not repaired (unlike the nucleus, mitochondria do not have all the proteins that repair any damaged DNA). All DNA generally has a low mutation rate, but mtDNA has a higher rate than nuclear DNA, so making it useful in evolutionary studies.

When the base sequences of mtDNA of closely related species are compared, there are very few differences. When they are compared with more distantly related species there are more differences. The number of differences between two species is used to assess how closely they are related and when the ancestors of the two species diverged and speciated (see page 201). As mtDNA is thought to mutate at a constant rate, the number of differences act as a molecular clock to date these divergences.

Sequencing **primary structures of proteins** shows that closely related organisms have sequences that are near identical. The sequences of proteins with the same function in unrelated organisms have greater differences. For example, the active site of an enzyme like catalase (see page 27) tends to be identical whatever organism it comes from, as no other arrangement of amino acids gives the right 3D shape to fit the specific substrate. These sequences are described as being highly conserved. Other parts of the enzyme molecule will show differences between different organisms: the fewer the number of differences, the more closely related the organisms.

Remember

Remember that chloroplasts also have DNA. cpDNA is also used in similar studies to find out how different plants are related to each other.

Remember

The structure of mitochondria is covered in Units 1 and 12. The primary structure of protein is given in Unit 2. Read these again if you are unsure about the principles involved in these two lines of evidence for evolution.

Natural selection

Charles Darwin and Alfred Russel Wallace proposed the theory of **natural selection** in 1858 to explain how organisms evolve. A year later Darwin published *On the Origin of Species* in which he described evidence for a way in which evolution could occur. He started by describing four general observations that he had made.

- **Overpopulation** – all species have the ability to produce large numbers of young individuals. Not all of these live to maturity.
- The populations of many organisms remain fairly **stable** from year to year with only minor fluctuations.
- There is considerable phenotypic **variation** within most species (intraspecific variation).
- Offspring have phenotypes that resemble those of their parents.

The theory of natural selection explains the role of the environment in determining which individuals survive to reproduce.

- There are limited resources available in the environment for each population. There is a **struggle for existence** as individuals **compete** for these resources. Animals compete for water, food, territories, nesting sites and mates; plants compete for space, water, ions, light, carbon dioxide and, in some cases, pollinators (e.g. insects). Individuals of the same species have the same means to obtain those resources.
- Competition between members of the same species for food leads to high death rates from starvation among young animals. They are also likely to be eaten by predators or die of disease. There is a similar high mortality among seedling plants that start growing in unsuitable places, do not absorb enough water, ions, light or carbon dioxide, are eaten by herbivores or are killed by disease.

Exam tip

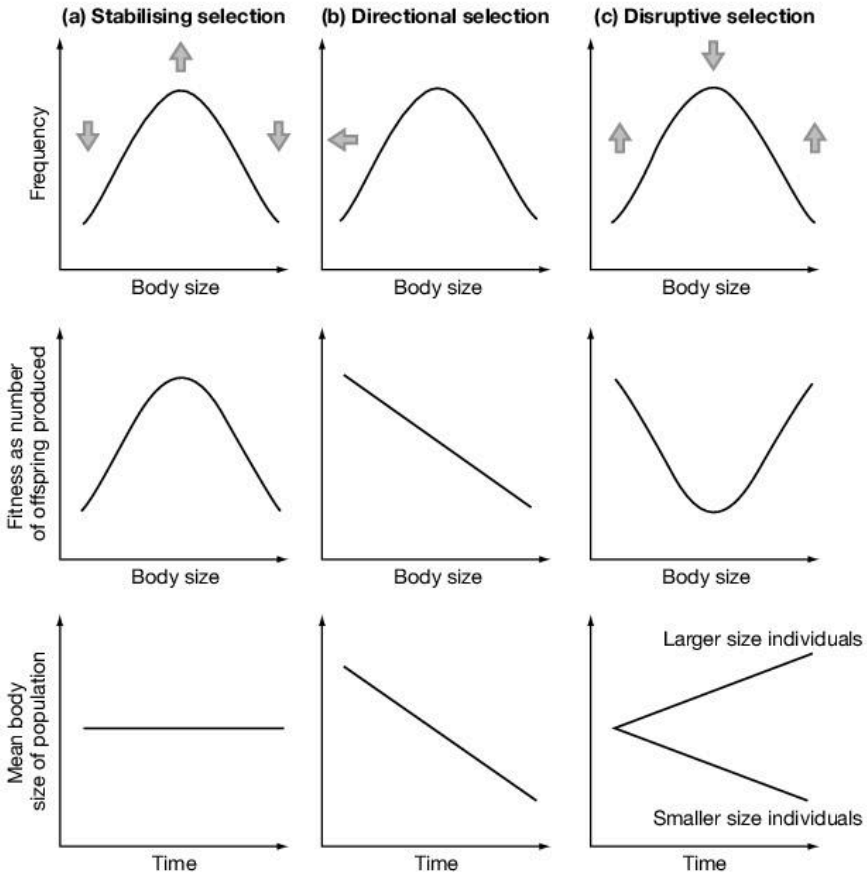
Competition between members of the same species is intraspecific competition – a useful term to use in an answer on natural selection.

Key term

Natural selection: the survival of individuals with particular features that adapt them to the factors of the environment, e.g. predators, competition and climate; these individuals have a greater chance of breeding and passing on their alleles than other individuals that are selected against.

- The individuals that are best **adapted** to obtain resources from their environment survive to reproduce and pass on their **alleles**. Those that do not survive to reproduce (or produce few offspring) and do not pass on their alleles are selected against.
- This **differential survival** means that populations mainly consist of individuals that are well adapted to the environmental conditions that exist at any one time.

Types of selection



▲ **Figure 17.4** These graphs show the effect of the three different types of selection on body size, a feature that shows continuous variation. Arrows show the direction of selection: left and up – positive selection; down – negative selection

Over the very long periods of time that life has existed on Earth, environments have tended to be stable. Populations do not change very much, something which is supported by studies of fossils in different strata of rocks. This type of selection is **stabilising selection**, in which there is selection against extremes. For example, the mean length of wings in bird species does not change between generations.

When the environment changes, the struggle for existence may become much harder for a population of a particular species. **Selection pressures** change and individuals with specific phenotypes that were selected against are now better able to find resources and survive. This was observed with ground finches on the Galápagos Islands following a severe drought. Only hard seeds were available to eat and only finches with larger beaks could eat them. As a result of this selection pressure, the mean beak size increased over several generations. This is an example of **directional selection**.

★ Exam tip

Notice that this says alleles and not genes. All individuals of the same species have the same genes – they differ in the alleles of those genes if they have two (diploid) or more (polyploid) sets of chromosomes. Some alleles give individuals the competitive edge that increases their chance of survival.

Key terms

Stabilising selection: favours intermediate forms in a population with selection against the extremes of the range. Allele frequencies remain constant (Figure 17.4a).

Directional selection: favours a rare form in a population; as a result the population changes over time as there is an increase in the frequency of some alleles (Figure 17.4b).

★ Exam tip

In an animal population, the individuals that survive to reproduce are good at finding food, escaping from predators, resisting disease and finding mates.

Environmental conditions sometimes change so that individuals at either end of the range for one or more particular features are at a **selective advantage** and those in the middle of the range are not. When this happens, it may give rise to two populations by **disruptive selection**. The two populations may change so much that eventually they cannot interbreed and are two separate species. For example, a bird predator feeds on medium-sized individuals of a species of freshwater snail so leaving larger and smaller snails to reproduce.

Key term

Disruptive selection: favours the extremes of a range in a population which may lead to the formation of two species; selection is against the most common form (Figure 17.4c).

Maths skills

Hardy–Weinberg principle

The Hardy–Weinberg principle is used to calculate the frequencies of alleles of a particular gene in a population. Equation 1 is for alleles and equation 2 is for genotypes:

Equation 1 $p + q = 1$

where p = the frequency of the dominant allele, **A**, (or whatever allele you are investigating) in the population; and q = the frequency of the recessive allele, **a**, in the population.

Equation 2 $p^2 + 2pq + q^2 = 1$

where p^2 = the frequency of the genotype **AA**; $2pq$ = the frequency of the genotype **Aa**; and q^2 = the frequency of the genotype **aa**.

In a population of mice, 16% have the recessive phenotype with the genotype **aa**. Using Equation 2, 16% becomes 0.16 which is equivalent to q^2. This means that we can calculate the frequency of the recessive allele (**a**) as the square root of 0.16 which is 0.4. Using Equation 1, the frequency of the dominant allele (**A**) is therefore 0.6 ($p = 1 - q$).

A modified Punnett square shows how equation 2 is derived if all individuals can mate *at random*.

		frequency of female gametes in the population	
		p (**A**)	q (**a**)
frequency of male gametes in the population	p (**A**)	p^2 (**AA**)	pq (**Aa**)
	q (**a**)	pq (**Aa**)	q^2 (**aa**)

We can now put these allele frequencies into Equation 2 and calculate the frequencies of the homozygous dominant genotype and the heterozygous genotype as **AA** = $0.6^2 = 0.36$ (36%) and **Aa** = $2 \times (0.6 \times 0.4) = 0.48$ (48%).

We can analyse the population in this way because the homozygous recessive individuals in the population have a phenotype that is recognisable. We cannot count the number of homozygous dominant individuals to find p^2 because they have a phenotype indistinguishable from that of the heterozygotes. We can use the equations to calculate the frequency of allele **A** in the population and therefore the frequencies of **AA** and **Aa**.

There are several requirements of the Hardy–Weinberg principle. It assumes:

- the population is large
- mating occurs within a population at random (mating in a small population is non-random (see page 200)
- no mutation
- no immigration or emigration
- no selective pressure operating against one of the phenotypes.

The Hardy–Weinberg equations can be used to see if allele frequencies change from generation to generation. If the frequencies of genotypes in one generation do not conform to the expected frequencies then one or more of the assumptions above does not apply. For example, It may be that selection is occurring so that the frequency of one allele is decreasing and the other is increasing.

Genetic drift

In small populations changes in allele frequency often occur at random rather than as a result of selection. It may be pure chance as to which individuals survive and breed when the population is small. Also mating is not at random as the numbers of individuals are so small. These changes in allele frequency are known as **genetic drift**.

There are two ways in which genetic drift occurs in small populations. In both cases there is considerable loss of genetic variation because of loss of alleles.

- a population is severely affected by some catastrophe (harmful event) so that there are very few survivors; the cheetah, *Acinonyx jubatus*, and the Northern Elephant seal, *Mirounga angustirostris* (Figure 17.5), are good examples.
- small populations that migrate and colonise new areas, especially islands or other isolated ecosystems, are likely to have allele frequencies that are not representative of the main population from which they came. This is the **founder effect**.

A genetic bottleneck is the term used to describe the effect of near extinction that occurred to species like the cheetah. Many species are near extinction now and if they do survive will suffer from a severe loss of genetic variation. This lack of variation makes them very susceptible to extinction if the environment changes.

The changes described so far apply to changes in populations of a species. According to the definition of the biological species (see Unit 18 page 207), these populations are all members of the same species because they can interbreed – they can reproduce with each other. With time a population may change significantly in other ways so that reproduction with other populations is not possible. Table 17.2 summarises the isolating mechanisms that act as barriers to reproduction between species.

▲ **Figure 17.5** A Northern elephant seal, one of the descendants of a very small population (about 20) that survived intense hunting at the end of the 19th century. Numbers have now recovered, but they only represent a small portion of the genetic diversity of the original population

Key terms

Genetic drift: the change in allele frequency that occurs by chance, not as a result of selection.

Founder effect: the loss of genetic diversity when a very small number of individuals colonise a new area.

▼ **Table 17.2** Isolating mechanisms that act as barriers to reproduction between species

Isolating mechanism	Explanation
Pre-zygotic – prevents fertilisation	
geographical	features such as rivers, lakes, mountains, lowlands, forests separate populations so they never or rarely meet
ecological	populations inhabit different habitats within the same area so individuals rarely meet
temporal	breeding occurs at different times
reproductive behaviour	courtship rituals are different so that males and females cannot mate
Post-zygotic – prevents the development of the embryo or the ability of the offspring to reproduce	
production of a hybrid prevented	fertilisation does not occur embryo fails to develop
breeding of hybrids prevented	offspring are sterile (often because homologous chromosomes cannot pair in meiosis) no viable individuals are produced

Speciation

Speciation is the process by which a new species arises from an existing species.

Allopatric speciation. If individuals of a species migrate to occupy a new area, such as an island, they are exposed to different selection pressures compared with the area that they have left. Over time this may lead to changes in the isolated population to the extent that they cannot interbreed with the original population.

Sympatric speciation. This occurs within a population without physical separation. Sometimes this is an abrupt change in a species, so that individuals are not able to interbreed.

Why do organisms become extinct?

Extinction is a fact of life on Earth. The main reasons for extinction are: climate change, competition, habitat loss and killing by humans. Often there is more than one cause of extinction.

Key terms

Allopatric speciation: formation of a new species when an original population becomes divided by a geographical feature and the separated population becomes isolated.

Sympatric speciation: formation of new species within a single population; an ecological or a behavioural mechanism results in two reproductively isolated populations within the same area.

Selective breeding (artificial selection)

★ Exam tip

To answer questions on selective breeding it helps to know something about how cereal crops reproduce (see formation of pollen in Unit 16 page 184) and how assisted reproduction occurs in cattle.

Crop improvement

Disease resistance in wheat and rice. Plant breeders have improved bread wheat, *Triticum aestivum*, by using techniques of selective breeding to incorporate the gene *Sr2*, responsible for resistance to stem rust disease, from the related species *T. turgidum* to produce a variety of bread wheat called Hope. *Sr2* has since been used in other varieties (but see Unit 19 page 229). Over 60 genes have been identified as improving resistance to disease in rice. Some of these act in the signalling pathway that activates the plant's defences against attack.

Gibberellin synthesis. Mutant alleles that do not code for the enzymes required to make gibberellins have been incorporated into varieties of cereal to give dwarf varieties. This has increased grain yield as dwarf varieties use less energy for growing and maintaining stems and leaves.

In this process of genetic improvement, a high-yielding commercial variety is crossed with a variety showing the desired characteristic. The seeds of the next generation are grown and then tested to see if they have the desired feature. These hybrids are then crossed with the high-yielding variety. This backcrossing occurs for several generations before the new variety is made available to farmers.

Inbreeding and hybridisation in maize. Breeders establish inbred varieties of maize that have some features they wish to incorporate, such as drought tolerance, by preventing cross-pollination and ensuring that only self-pollination occurs. After several generations plants become homozygous for most of their genes. The yield of these plants is low, but when they are crossed with another inbred line, the heterozygous offspring are highly vigorous, producing good yields of maize. This is known as **hybrid vigour**.

★ Exam tip

Search online to read more about how specific organisms have become extinct. Distinguish between local extinction and total extinction.

★ Exam tip

Make sure that you can distinguish between selective breeding (artificial selection) and genetic engineering.

★ Exam tip

Make a table to compare artificial selection (selective breeding) and natural selection to make sure that you understand the relevant similarities and differences.

Exam tip

You might be tested on your knowledge of meiosis by being asked why maize farmers cannot keep their seed from one year to the next. Instead, they have to buy seeds of hybrid corn from seed merchants. Think of how meiosis generates variation and the effect that this will have on subsequent crops. They may flower at different times and grow to different heights making harvesting very difficult.

Improving the milk yield of cattle

The milk yield of dairy cattle is a feature that is influenced by environmental factors, such as quantity and quality of feed, and genetic factors. Yields have increased over the past 60 years or so by providing high quality feed, controlling disease and providing shelter so that more energy can be devoted to making milk. Herds have also been improved by selective breeding and assisted reproduction, such as artificial insemination (AI), IVF and embryo splitting to produce clones.

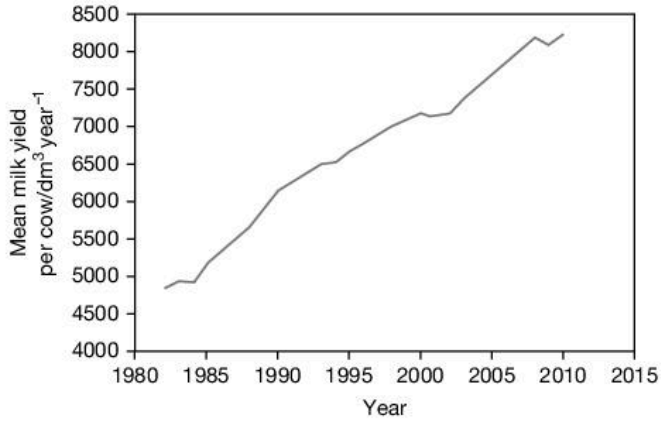
Cows that produce large quantities of milk are inseminated with sperm from a high-quality bull that is known to have female offspring that produce high volumes of milk. By using AI, sperm from one bull may fertilise eggs in thousands of cows. Eggs from cows that show superior qualities may be harvested, fertilised *in vitro* and the embryos placed in surrogate cows. This protects the superior cow from the risks of pregnancy.

Exam tip

Breeders of dairy cattle also select for other desirable features, such as docility (not aggressive), udder shape and high food conversion. You should read about the selective breeding of other livestock to make sure you can write about this topic with confidence.

Exam tip

Distinguish carefully between artificial insemination (AI) and *in vitro* fertilisation (IVF). AI involves collecting sperm from superior males, storing it and then placing samples into the reproductive tracts of cows so fertilisation occurs in the female. In IVF, eggs are collected and then fertilised outside the animal, in a lab.



▲ **Figure 17.6** The increase in milk yield in cows in France, because of genetic improvement

Raise your grade

1 (a) Describe two examples of the effects of the environment on the phenotype of named organisms. [2]

Animals will put on weight if they eat too much and may be burnt by the sun if it is very hot.

The candidate has not read the question carefully. There should be a named organism in the answer not just 'Animals'.

(b) Explain how the interaction of alleles can influence the phenotype of an organism when
(i) the alleles are at one locus
(ii) the alleles are at different loci. [5]

The alleles of a gene can be dominant, recessive or codominant. These can have important effects on the phenotype because sometimes the allele has an effect (dominant) and sometimes it does not (recessive). Both codominant alleles have an effect at the same time. ✔

A poor answer that should have detail about the interaction of dominant and recessive alleles. Recessive alleles have an effect on phenotype. This does not attempt an answer to part **(ii)**. This shows the importance of knowing the syllabus so that you can anticipate the questions.

(c) The mean milk yield for cattle in the UK has increased from 4099 dm^3 per cow per year in 1975 to 7557 dm^3 per cow per year in 2017.
Explain how a cattle breeder can increase the milk yield of a herd of dairy cattle. [5]

The best cattle are crossed together producing cattle that will produce better milk. Then they will take the best cattle from the offspring and breed them together. And so on.

A very poor answer that overlooks the fact that male cattle (bulls) do not produce milk.

2 Cystic fibrosis (CF) is the most common genetic disease in Caucasian populations. In these populations, 1 in 2500 babies are born with the condition.

(a) Use the Hardy–Weinberg principle to calculate the proportion of these populations that are carriers of the mutant allele, **f**, for cystic fibrosis. [4]

p is the frequency of the dominant allele (**F**) and q is the frequency of the recessive allele (**f**). As 1 in every 2500 people are affected, the recessive allele $q = 0.0004$ and the dominant allele $p = 0.9996$. So the frequency of allele **f** = 0.0004 and that of **F** = 0.9996 in the population.

Inserting these frequencies into the equation: $p^2 + 2pq + q^2 = 1$ ✔
So the frequency of carriers (**Ff**) $= 2pq = 2 \times (0.0004 \times 0.9996) = 0.000799 = 0.08\%$ ✔

This answer contains a serious error in the first step. The frequency of children with CF is $1/2500 = 0.0004$, but that is q^2 and **not** q. The frequency of allele **f** is the square root of 0.0004 which is 0.02. So $p = 0.98$ and the frequency of carriers (**Ff**) is $2pq = 2 \times (0.98 \times 0.02) = 0.0392$ or 3.92% of the population. A mark can be awarded for calculating $2pq$ using the incorrect figures for p and q.

(b) The Ngorongoro crater in northern Tanzania is relatively isolated from the neighbouring Serengeti National Park. In the early 1960s, the population of lions in the crater numbered about 70. In 1962, the lions suffered a serious disease, reducing the population to nine females and one male. Seven more males migrated into the area and this population remained isolated for at least the next 25 years, recovering to between 75 and 125 animals. A study of the lion population in the crater in the late 1980s showed low genetic diversity, low reproductive rates and high proportions of abnormal sperm in the males' semen.

(i) Suggest the information that needs to be collected to make an assessment of the genetic diversity of a population. [3]

Identify the number of different alleles for each ✔ gene and then find out their frequencies in the population ✔. Also find out how many lions are heterozygous ✔ for each gene.

This answer makes three correct ideas very concisely. It would involve a lot of work if all the lions were to be assessed. This sort of work is more likely to be done on a sample of the lions, not the whole population. In the 1980s, genetic diversity was assessed by looking at a small number of genes. DNA sequencing now allows the collection of much more data on genetic diversity (see page 226).

(ii) Explain why the lion population had such a low genetic diversity even though numbers had recovered from the crash in the 1960s. [3]

As there were only 17 lions left to breed, there cannot have been much genetic diversity amongst them and alleles of some genes would not be present. Inbreeding has occurred. ✔ Males and females are closely related so over time many in the population become homozygous for many genes. ✔ Changes in allele frequencies are likely to be due to genetic drift (non-random mating), rather than to natural selection. ✔

The candidate has the right ideas. Genetic drift is involved. This is an example of a genetic bottleneck rather than a founder effect as the population after the disease was not composed entirely of migrants from another area, such as the Serengeti.

Exam-style questions

Structured questions (Paper 4)

1 In 2004, Hurricane Frances completely destroyed populations of the brown anole lizard, *Anolis sagrei*, on seven small islands in the Bahamas.

(a) Outline **two** other ways in which organisms can become locally extinct. [4]

A research team re-established small populations of brown anoles on these islands. The team collected seven males and seven females from a population on a larger island, Iron Cay. These lizards were chosen completely at random. A pair of lizards was released on each island.

Brown anoles perch on the branches of small trees. The trees on Iron Cay have wider branches than those on the small islands and favour lizards with long hind limbs. The introduced pairs all had long hind limbs.

The pair of lizards introduced to each island bred successfully and had offspring that showed variation in the length of the hind limbs.

After three years the mean length of the hind limbs of all the lizards on the small islands was significantly less than the mean of the lizards on Iron Cay. A study of the allele frequencies for selected genes showed that there were significant differences between the populations on each of the small islands.

(b) Suggest and explain why

(i) the mean hind limb length of the lizards had changed [5]

(ii) the allele frequencies were different between the populations of lizards on the seven islands. [3]

2 (a) Explain why body mass is an example of continuous variation. [2]

Three separate populations of mice on different islands across the world were studied over a long period of time. The researchers collected data on body mass. At the beginning body mass of all the mice showed a normal distribution and the mean body mass of each population was about the same.

At the end of the study, these results were obtained:

Population **A** the mean body mass remained the same, but the range in mass decreased

Population **B** the mean mass increased, but with fewer small mice and more larger mice

Population **C** there were many small mice and many much larger mice, but none with the mean mass of the original population.

(b) Draw graphs for populations A, B and C to show the variation in body mass at the beginning and at the end of the study. [7]

(c) Name the type of selection that occurred to each population. [3]

Free response questions (Paper 4)

3 (a) With reference to a named organism, discuss the differences between continuous and discontinuous variation. [8]

(b) Outline why genetic variation is important in populations where natural selection is acting. [7]

4 (a) Explain the roles of extinction, geographical isolation and small population size in the evolution of new species. [10]

(b) Discuss the advantages of using mitochondrial DNA to provide evidence for evolution. [5]

extra questions available online

18 Biodiversity, classification and conservation

Key points

- ☐ Define the terms species, ecosystem and niche.
- ☐ Explain that biodiversity is considered at three different levels: variation in ecosystems or habitats; the number of species and their relative abundance; and genetic variation within each species.
- ☐ Explain the importance of random sampling in determining the biodiversity of an area.
- ☐ Use suitable methods to assess the distribution and abundance of organisms in a local area.
- ☐ Use Simpson's Index of Diversity (D) to calculate the biodiversity of a habitat and state the significance of different values of D.
- ☐ Use Spearman's rank correlation and Pearson's linear correlation to analyse the relationships between the distribution and abundance of species and abiotic or biotic factors.
- ☐ Describe the classification of species into the taxonomic hierarchy of domain, kingdom, phylum, class, order, family, genus and species.
- ☐ Outline the characteristic features of the three domains Archaea, Bacteria and Eukarya and of the kingdoms Protoctista, Fungi, Plantae and Animalia.
- ☐ Explain why viruses are not included in the three-domain classification and outline how they are classified.
- ☐ Discuss the threats to the biodiversity of aquatic and terrestrial ecosystems and discuss the reasons for the need to maintain biodiversity.
- ☐ Discuss methods of protecting endangered species, including the roles of zoos, botanic gardens, conserved areas (national parks and marine parks), 'frozen zoos' and seed banks.
- ☐ Discuss methods of assisted reproduction, including IVF, embryo transfer and surrogacy, used in the conservation of endangered mammals.
- ☐ Discuss the use of culling and contraceptive methods to prevent overpopulation of protected and non-protected species.
- ☐ Use examples to explain the reasons for controlling alien species.
- ☐ Discuss the roles of non-governmental organisations in local and global conservation.
- ☐ Outline how degraded habitats may be restored with reference to local or regional examples.

Biodiversity

An ecosystem is a relatively self-contained unit, made up of biotic and abiotic components interacting and functioning together. An ecosystem includes the community of living organisms of all types in a given area and all the abiotic (physical and chemical) factors in their environment, linked together by energy flow and cycling of nutrients. Ecosystems may vary in size but always form a functional entity: for example, a decomposing log, a pond, a meadow, a coral reef, a forest, or the open ocean. The environment provides habitats for different **species**. Each species within an ecosystem occupies a particular **niche** that it does not share with any other species. The niche occupied by any species is determined by the effect of biotic and abiotic factors in the ecosystem.

★ Exam tip

A habitat is a place where a particular species lives and a community is all the populations of all the species in a given area at a given time. The term niche incorporates the habitat and all the interactions that a species has with other organisms and its physical environment.

A Level

The biodiversity of an area, such as a country or a region, is a measure of:

- the different ecosystems and different habitats (aquatic and terrestrial)
- the number of different species
- the abundance of each species, and
- the genetic diversity within each species.

Tropical forests and coral reefs are two of the most species-rich areas on Earth.

Key terms

Species: a group of organisms that share the same features and can reproduce together to produce fertile offspring that are reproductively isolated from other species.

Niche: the role of a species within a community including its position in the food web and its interactions with other species and its environment.

Biodiversity: the variety (diversity) of ecosystems, habitats, species and the genetic diversity within species that exists in an area.

Species diversity is considered important as it makes an ecosystem more able to resist changes than one with limited diversity. Generally, the further north you go from the equator, the more species diversity decreases.

Genetic diversity within a population considers factors that give different genetic characteristics, such as the number of different alleles in the genome and the number of loci that have multiple alleles. Different populations of the same species may be adapted differently, so could vary in their genetic diversity. Genetic diversity allows populations to adapt to changing environmental conditions.

Sampling biodiversity

To overcome any bias, **random sampling** is used to make sure that results are representative of a whole area. An area is marked out with tape measures to make a grid; random numbers are used to give coordinates and sampling is done where the coordinates intersect. Sampling communities is usually done with a quadrat:

- an open frame quadrat is a square frame made of wood, plastic or metal enclosing a known area – usually 0.25 m^2 or 1.0 m^2; larger areas can be marked out with tapes or string
- a gridded quadrat is a frame quadrat divided into smaller sections (e.g. 10 × 10). Random samples within a small area can be taken rather than counting individual organisms in the whole quadrat.

A quadrat can be used to record the species that occur within it and then calculate **species frequency** – the percentage of quadrats that contained each species.

The abundance of different species may be recorded by:

- counting individual plants and animals where this is possible and expressing results as **species density** – number of individuals per unit area
- estimating **percentage cover** (e.g. for organisms that are difficult to count individually) by finding the percentage of a quadrat that is occupied by each species
- using an **abundance scale** when species are far too numerous to count and, for plants, when it is difficult to isolate individuals (e.g. ACFOR).

Exam tip

Make sure that you know the difference between the distribution of a species and the abundance of a species. Distribution is where the species occurs in an area – the spread of a species. Abundance is a measure of how many organisms of that species there are in a given area and can be in given terms of numbers, biomass or percentage of the area covered.

Link

There is more about genetic diversity in Units 17 and 19 – see pages 194 and 226. You may be asked about the techniques to investigate genetic diversity (Unit 19) and the importance of genetic diversity for natural and artificial selection.

Remember

You can illustrate answers on genetic diversity with examples from other Units. Sickle cell anaemia (see Units 6 and 17) is an example of genetic diversity. In fact, it is estimated that there are about 900 different variants of the haemoglobin polypeptides in the human population – all with slightly different amino acid sequences.

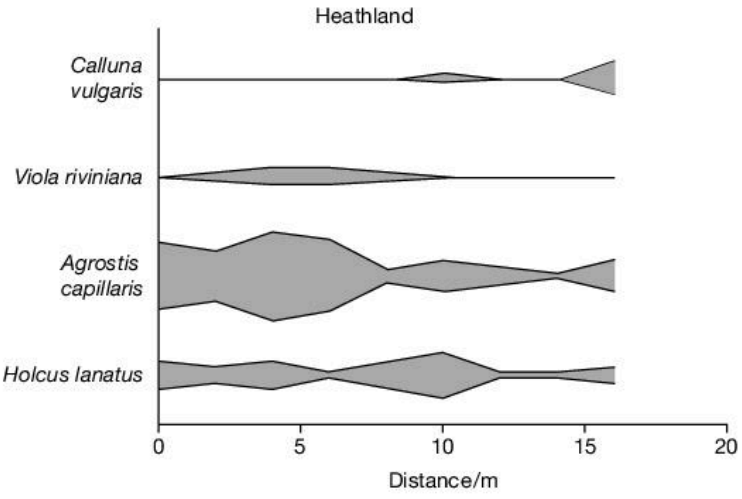
Random sampling using quadrats is usually done in a uniform ecosystem, such as grassland or woodland. It is not appropriate if the physical environment is not the same across the whole area being sampled.

Exam tip

Some biodiversity surveys are carried out in much larger areas. For example, ecosystem diversity in the Amazon region was assessed with quadrats that were 1000 km by 1000 km.

A **line transect** is useful for assessing non-uniform distribution, for example, an area where there is an abrupt change from one ecosystem to another, such as between grassland and a woodland. A long rope or tape measure marked at intervals is placed across an area. The species at each sampling point along the transect is recorded and the results used to make a drawing that shows the changes in **distribution** of species. Line transects are used to show how communities change along a gradient, which could be a slope or a change in an abiotic feature, such as exposure to the wind or change in soil moisture.

A **belt transect** is used to collect quantitative data along a transect. Quadrats are placed along the side of a line and used to assess species abundance in a **continuous belt transect**. Placing quadrats at intervals (e.g. every 5 m) gives an **interrupted belt transect**. Data may be collected as percentage cover and species density. Data from a belt transect may be used to make a kite diagram (Figure 18.1).



▲ **Figure 18.1** A kite diagram which shows the distribution and abundance of four plant species across an area of heathland

Measuring abiotic factors

The distribution of species throughout ecosystems is determined by biotic and abiotic factors. Data on abiotic factors can be collected at sampling points along transects. Factors may include light intensity; temperature; humidity of air (measured with a hygrometer); wind speed (measured with an anemometer); oxygen concentration, pH and salinity of water; and pH and water content of soil.

Abiotic factors must be collected consistently, for example, at the same height or depth. The results for the abiotic factors may be written on kite diagrams to see if there is any correlation. If this seems likely, then correlation coefficients can be calculated (see page 210).

ACFOR – abundant, common, frequent, occasional, rare. Decide first how to apply each description; how much or how many plants or animals have to be present before you can record their abundance as 'common' or 'frequent', for example. Then you can make your recordings far quicker than making counts.

Key terms

Quadrat: apparatus for analysing composition of a community, e.g. a frame quadrat

Transect: a tape or rope placed across an area so that samples are taken at regular intervals, e.g. 0.5 m.

Line transect: the organisms that touch the transect are recorded to show species distribution.

Belt transect: quadrats placed at intervals along a transect are used to record the abundance of organisms.

Exam tip

Look at the section on biological sampling under the Education tab at the Offwell Woodland & Wildlife Trust web site. There are plenty of examples of sampling techniques to read about.

If field work is carried out on several occasions, then information about changes over time can be collected. Sensors and data loggers can be used to record abiotic factors over the long term.

Mark-release-recapture

One way to estimate numbers of a mobile population of animals is to catch a certain number and mark them – the first sample (S_1). Release these animals back into the environment and later catch a second sample (S_2). S_2 should contain both marked and unmarked individuals. The smaller the proportion of marked individuals, the larger the total population. Calculate an estimate of the population size by using the **Lincoln index** (also known as the **Petersen index**):

$$N \text{ (population size)} = \frac{n1 \times n2}{m2}$$

where N = population estimate; $n1$ = number of marked individuals released; $n2$ = number of individuals (both marked and unmarked) captured; and $m2$ = number of marked individuals recaptured.

▲ **Figure 18.2** Students measuring the infiltration rate – the speed at which a known volume of water enters a known area of soil. They compared the rates for soils in a palm oil plantation with soils in a rainforest

Maths skills

Simpson's Index of Diversity

Expressing species diversity as one figure is simpler than looking at lists of species found in different ecosystems and their abundance. **Simpson's Index of Diversity** uses the number of species and their relative abundance to calculate a value between 0 and 1. The higher the number the greater the diversity (D).

The formula for calculating D is: $D = 1 - \sum \left(\frac{n}{N}\right)^2$

where $\sum$ = the sum of; n = the number of each organism recorded; and N = the total number of all organisms.

This index measures the probability that two individuals randomly selected from a sample will belong to the same species, or group (e.g. genus or family).

★ Exam tip

There are many different indices of diversity and at least two ways to calculate the Simpson's index. Learn the steps to follow when calculating this version.

Worked example

Many plants that grow in waste ground can be counted as individual plants. Random sampling is used as counting all the plants in an area is too time-consuming. Some students studied the vegetation on an area of waste ground in Barbados. They recorded their results as follows:

thick-leaved grasses 40, thin-leaved grasses 150, Pride of Barbados 3, heart seed 5, Mexican poppy 18, wild cress 15, wild dolly 11, black sage 17.

(a) Use the formula above to calculate Simpson's Index of Diversity.

(b) State a conclusion that the students could make from their calculation.

(c) State **one** limitation of the data that the students collected.

★ Exam tip

You should expect to plan a field work investigation in Paper 5 using the techniques of random or non-random sampling.

Answers

(a) $D = 1 - 0.3511 = 0.6489$

The Index of Diversity (D) is 0.65 (to 2 significant figures).

The working in the answers to the questions for this unit are available on the support website.

(b) When the index is small (near 0) there is a very low diversity. When the number is high (near 1) there is a very high diversity. This habitat is dominated by the thin-leaved grasses so does not have a very high diversity.

(c) They did not identify different species within the thin-leaved and thick-leaved grass species.

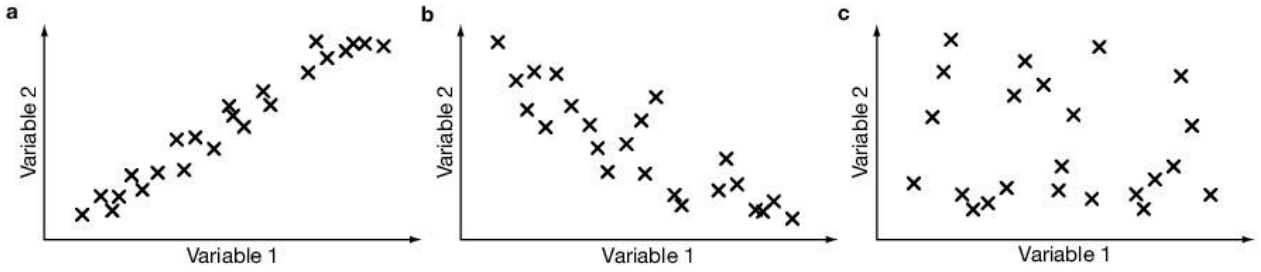
Correlations

If your field work involves investigating the relationship between the distribution and/or abundance of two species or the effect of an abiotic factor on the distribution and/or abundance of a species, then you should calculate a correlation coefficient.

The relationship between two variables, such as the abundance of species A and species B, may be positive, negative or non-existent, as shown in the scatter graphs in Figure 18.4.

★ Exam tip

If in doubt about which correlation coefficient to calculate, use Spearman's rank, but there are occasions when Pearson's linear is more appropriate.



▲ **Figure 18.4** Scatter graphs: a positive correlation; b negative correlation; c no correlation

The strength of the correlation can be assessed by calculating a correlation coefficient. A coefficient of +1 indicates there is a perfect positive correlation (all the points are on a straight line) and −1 indicates that there is a perfect negative correlation. A coefficient of 0 means the points are scattered at random on the graph and there is no correlation.

★ Exam tip

The scientific name for each species is *both* names. The second name should not be used on its own.

Classification

Every known species has two unambiguous scientific names. The first or genus name begins with a capital letter and the second begins with a lowercase letter, e.g. *Taraxacum officinale* for the dandelion plant. When a scientific name has been used once, it may be shortened, e.g. *T. officinale*.

Classification is the organisation of living things into groups that are arranged in a hierarchy. The hierarchical classification system has large groups which are continually subdivided to the level of the species.

▼ **Table 18.3** The hierarchical classification of three species of mice

Taxonomic rank	Wood mouse	House mouse	Macleay's marsupial mouse
Domain	Eukarya	Eukarya	Eukarya
Kingdom	Animalia	Animalia	Animalia
Phylum	Chordata	Chordata	Chordata
Class	Mammalia	Mammalia	Mammalia
Order	Rodentia	Rodentia	Dasyuromorphia
Family	Muridae	Muridae	Dasyuridae
Genus	*Apodemus*	*Mus*	*Antechinus*
Species	*sylvaticus*	*musculus*	*stuartii*

Exam tip

Table 18.3 shows the hierarchical arrangement of the taxonomic ranks beginning with the domain and ending with species. Each of the groups in the rest of the table (e.g. Eukarya, Rodentia, etc.) is a taxon (plural: taxa). The wood mouse and the house mouse are more closely related to each other than to the marsupial mouse.

▲ **Figure 18.5** *Antechinus stuartii* is a marsupial mouse from Australia. It is carnivorous — unlike the other two species in Table 18.3. Superficially it looks like a mouse, but it is not closely related to the other two species

The three domains

The key features of the three domains are concerned with DNA, ribosomes and RNA polymerase. The Archaea share features with the other two domains, but are considered to be a distinct group of prokaryotic organisms (Table 18.4).

▼ **Table 18.4** The features of the three domains

Feature	Domains		
	Bacteria	**Archaea**	**Eukarya**
cell structure	prokaryotic (see Unit 1)	prokaryotic	eukaryotic
nucleus	✘	✘	✔
DNA	circular	circular	linear
DNA with histones	✘	✔	✔
plasmids	present in many	present in some	none (rare exceptions, e.g. yeasts)
ribosomes	all 70S	all 70S; structurally different to bacteria and share some features with Eukarya	all in the cytosol are 80S (70S in mitochondria and chloroplasts)
peptidoglycan (murein) in cell wall	✔	✘	✘
method of cell division	binary fission	binary fission	mitosis
organisation	single-celled or chains / groups of cells, e.g. filaments	single-celled or chains / groups of cells, e.g. filaments	unicellular / colonial / multicellular

▼ **Table 18.5** Some of the main features of the five kingdoms

Features	Protoctista	Fungi	Plantae	Animalia
Type of body	unicellular and multicellular	mycelium composed of hyphae; yeasts are unicellular	multicellular, not compact	multicellular, most have a compact body
Cell walls	present in some species	✔ (most made of chitin)	✔ (made of cellulose)	✘
Type of nutrition	autotrophic and heterotrophic	heterotrophic (saparotrophic or parasitic)	autotrophic	heterotrophic
Motility (ability to move themselves)	some protoctists have flagella or cilia or pseudopodia	✘	✘	✔
Nervous coordination	✘	✘	✘	✔
Examples	*Amoeba*, algae, slime moulds	yeasts, mould fungi (e.g. *Aspergillus* and *Penicillium*)	liverworts, mosses, ferns, conifers, flowering plants	jelly fish, coral, worms, insects, vertebrates

Viruses are not included in the three domain classification. Each virus is composed of nucleic acid surrounded by a protein coat. As they do not have a cellular structure and have no form of metabolism, they cannot be classified with prokaryotes and eukaryotes. They are classified according to the type of nucleic acid (RNA or DNA) and whether the nucleic acid is single stranded or double stranded.

Link

See Units 1 and 10 for more about viruses. A question on their classification may be set in the context of their role in disease and also ask for comparisons with prokaryotes and eukaryotes.

Exam tip

Viruses are the ultimate parasites, they cannot replicate or spread without invading the cells of prokaryotes or eukaryotes. The host cells produce more viral particles that are released and spread to infect more cells.

Conservation

Threats to biodiversity

The four main threats are:

- **Human population growth**; more humans means more land needed for housing, agriculture, industry, infrastructure, transport and recreation so reducing the space available for natural ecosystems.
- **Monocultures of crops**, such as wheat, maize and rice; arable agriculture removes natural vegetation (so reducing ecological niches) and uses fertilisers and pesticides, which damage the wildlife that can survive in these often hostile, uniform environments.
- **Pollution from human activities** (e.g., combustion of fossil fuels, disposal of untreated waste, waste from cruise ships).
- **Climate change** is happening at a rate that is more significant than would occur naturally because of human influences affecting the distribution and abundance of species, especially small populations (e.g. on isolated islands), those already under threat from disease (e.g. amphibians) and those with specific habitat requirements (e.g. alpine plants that live at high altitudes).

There are many reasons for needing to maintain biodiversity.

Religious/cultural beliefs: human responsibility in protecting the environment; religious belief in protecting other organisms.

Ethical: correcting damage already done by humans; maintenance of wild places for future generations to enjoy.

Technological: useful products such as the heat-stable enzyme, *Taq* polymerase; bacteria used to extract valuable elements, such as copper.

Unknown medicinal benefits: antibiotics are isolated from fungi and bacteria; anti-cancer drugs have been isolated from plants; plants used in Chinese medicines may provide drugs that can be mass produced.

Social/cultural/aesthetic benefits: for example, amateur ornithologists and botanists who enjoy wildlife; the natural world provides inspiration for artists, photographers, poets, writers and other creative people.

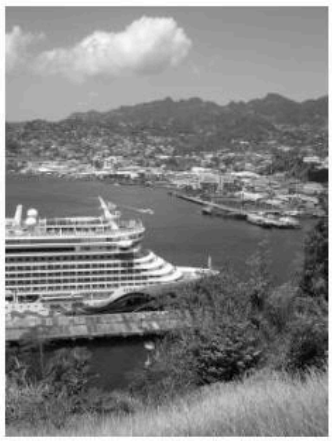
Economic benefits: ecotourism has increased in popularity.

Potential future environmental change: wild relatives of organisms can provide the genetic resources that we might need to widen the genetic diversity of cultivated organisms threatened by adverse conditions.

Indirect value: ecosystems provide benefits; for example, reefs and mangrove forests protect coasts from erosion.

Exam tip

An aquatic ecosystem is found in a body of water. The water in marine ecosystems, such as the open ocean and coral reefs, has much higher concentrations of solutes than in fresh water. Examples of freshwater ecosystems are ponds, rivers and lakes.



▲ **Figure 18.6** Cruise ships are a threat to marine and terrestrial habitats. Conservation organisations accuse cruise ship owners of polluting the seas and the pressure of tourists on small islands can be considerable

Exam tip

A question on this topic could include data on differences in biodiversity between marine and freshwater ecosystems or the effects of pollution on one or both ecosystems.

Methods of protecting endangered species

Conservation of species in their natural habitats (*in situ*) – national parks and marine parks

This is the best way to conserve a species as all the 'life support' systems are provided.

Exam tip

The web site of the International Union of Nature Conservation will tell you about the conservation status of many species. Try using the web site to research some endangered and vulnerable species from your country.

In situ conservation also involves enforcing measures in areas that are not designated as special areas; for example, reclaiming ecosystems that have been damaged by human activities and by natural catastrophes, such as volcanic eruptions, hurricanes and flooding; and preventing pollution damaging ecosystems.

Exam tip

Equally important are ecosystems threatened by development; the most well-known are tropical rainforests.

Conservation *of species ex situ*

Some species are so threatened in their habitat that they are removed to be kept somewhere else.

Zoos. Their role in conservation includes protecting endangered and vulnerable species using captive breeding programmes (e.g. for cheetahs); reintroduction programmes; researching their biology; and educating the public about wildlife and conservation.

Exam tip

Genetic diversity in cheetahs is very low because they nearly became extinct 10 000 years ago and only a few survived. All living cheetahs are descended from these individuals. Maintaining genetic diversity is an aim of conservation of many species.

Exam tip

Any method of conservation that keeps whole organisms, gametes, embryos, seeds, tissues or any other part of an organism is known as a **gene bank**. It is not a store of bits of DNA but of whole genomes in one of the ways listed.

Frozen zoos. These are gene bank facilities for storing sperm, eggs, embryos and cell cultures from many endangered and vulnerable species. Cooperative breeding programmes generate genetic diversity so species do not become inbred.

Botanic gardens. Their roles include: protecting endangered plant species; researching methods of reproduction and growth; researching conservation methods; reintroducing species to habitats where they have become very rare or locally extinct; and educating the public in the many roles of plants in ecosystems and their economic value.

Seed banks. Seeds are put into long-term storage in botanic gardens and research institutions by dehydrating or freezing them. Their viability is checked periodically. Some seeds that do not survive dehydration and freezing (e.g. cocoa) are harvested and resown in a field gene bank. Any environmental disasters would put these species at great risk of reduced genetic diversity.

Overpopulation

In situ conservation can sometimes result in species becoming a risk to the biodiversity of an ecosystem due to overgrazing, habitat destruction or, for plants, outcompeting rare species. For animals, one solution to this is culling.

Key term

Culling: the selective killing of individual animals to reduce the size of the population.

Another solution is to use contraception. Oestrogen implants can prevent the oestrous cycle in mammals. A vaccine against a glycoprotein in the zona pellucida, the layer surrounding the eggs (oocytes), produces antibodies in the female against her own oocytes so they do not develop into embryos, and so fail to implant.

Assisted reproduction

Eggs are fertilised *in vitro* and the zygotes divide to form small embryos. The embryos are then frozen until a surrogate mother becomes available. The embryos are inserted in the uterus of the surrogate mother. This technique of embryo transplantation has been used for many species including wild ox and different species of African antelope.

Exam tip

Do not confuse artificial insemination (AI) with *in vitro* fertilisation (IVF). In AI the sperm are placed inside the female's reproductive tract, so fertilisation is internal. IVF occurs outside the body in a lab.

Key terms

Assisted reproduction: any technique that is involved in treating infertility or protecting a valuable or rare female mammal from the health risks of pregnancy.

***In vitro* fertilisation**: the fertilisation of an egg that occurs outside the body of a female, e.g. in a Petri dish.

Embryo transfer: multiple embryos are removed from the uterus of a valuable or rare female mammal shortly after fertilisation and transferred to surrogate females to bring to full term.

Surrogacy: a female becomes pregnant with an embryo from another female and carries it to full term. Embryos can be conceived naturally, by AI or by IVF.

Alien species

Red lionfish, *Pterois volitans* – voracious predators that originate in the Far East – have quickly spread throughout the Caribbean where they outcompete native fish and are having a detrimental effect on fish biodiversity. There are programmes in places such as Belize that use divers to spear lionfish to reduce their populations.

The Galápagos Islands have been severely affected by human activities. In the past, whalers killed huge numbers of marine mammals. Many mammals (e.g. pigs, dogs) and plant species were introduced to the islands, which has had a detrimental effect on indigenous (native) species.

International conservation

The *Convention in International Trade in Endangered Species of Wild Fauna and Flora (CITES)* is an international treaty that protects animals and plants from various forms of exploitation, for example, illegal trade in animals for the pet trade and in animal materials, such as ivory.

The World Wildlife Fund for Nature (WWF) is a non-governmental organisation (NGO) that provides funding for conservation and environmental projects in many countries and runs campaigns on the behalf of wildlife and conserving wild places.

Link

Hormones are also used in the control of the menstrual cycle in humans (see Unit 15 page 179).

Link

See Question 2 on page 217 for contraception in elephants.

Exam tip

If you know of any alien species in your country, find out where they came from and the reasons for controlling them. If not, read about cane toads, water hyacinth, Japanese knotweed and kudzu.

Exam tip

Find out more about how the WWF and CITES are acting in your country on a national and global level.

Raise your grade

1 The giant river otter, *Pteronura brasiliensis*, has a range that extends across South America, living along the Amazon River, its tributaries and in the Pantanal swamp. The International Union for Conservation of Nature (IUCN) classifies the giant river otter as endangered. Numbers decreased partly as a result of hunting for its fur. Trade in otter skins was banned in 1975, so removing one threat to its survival.

(a) The giant otter is a top carnivore in the Pantanal. Explain why it is important for the biodiversity of ecosystems that top carnivores are conserved. [3]

Top carnivores help to control numbers of species below them in the food chain. This prevents herbivores ✔ overgrazing ✔ and leading to the extinction of competitors and plant species. They help to maintain the biodiversity and also the stability of ecosystems ✔.

The candidate could also have added that predators tend to take old and sick individuals which helps to maintain healthy populations of herbivores.

(b) Explain the risks to a species of hunting and overexploitation. [4]

Small population size makes it likely that the species will become extinct ✔. Before that the population will be small and it doesn't take much for all of them to be wiped out. If this doesn't happen, then the reduction in numbers inevitably means a reduction in genetic diversity ✔ as alleles of many genes are lost from the gene pool. Non-random mating between individuals ✔ is likely to cause any rare recessive alleles to be expressed which were not before ✔. These could be harmful in homozygotes, maybe even lethal.

Notice the correct use of terminology from Unit 17 (gene pool, loss of alleles). In addition, the candidate could have said that genetic drift might occur.

(c) DNA analysis suggests that there are four genetically distinct groups of giant river otters. Discuss the consequences of these results for conservation of the giant river otter. [3]

These four groups represent the genetic diversity of this species ✔. To maintain genetic diversity for recovery of the species, all four types of otter will have to be conserved ✔. This will involve protecting a larger area than if all giant otters throughout their range were genotypically the same ✔.

The candidate could discuss natural selection and adaptation to local conditions - after all the giant river otter lives across a vast area and river ecosystems may be different to swampland in the Pantanal.

(d) The otter in the photograph lives in Budapest Zoo in Hungary. Outline the arguments for and against keeping animals, such as *P. brasiliensis*, in zoos. [5]

Animals in zoos are protected against the threats in their natural habitat and this gives time for their habitats to be restored ✔ so that they can reintroduced. They can be studied carefully and zoos can try captive breeding ✔.

The answer could be improved by referring to education. No arguments against zoos have been given. The point about reintroduction could be developed much more in the answer. Read the IUCN Red List entry for the giant river otter. It will give you many ideas about how to write answers on the threats to terrestrial and aquatic habitats.

(e) The Pantanal swamp is a huge wetland extending over nearly 200 000 km^2 in Brazil, Bolivia and Paraguay.
Discuss the roles of international organisations in the conservation of areas of global importance, such as the Pantanal. [5]

If trade is banned by CITES then poaching should decrease giving populations of otters a chance to recover ✔. WWF is an important international organisation that could put pressure on countries to stop loss of habitat in the Pantanal, such as drainage and deforestation ✔. WWF can also fund research and/or habitat protection schemes ✔.

The syllabus lists CITES and WWF so it is best to refer to them if possible, but if you know of others then you should write about them.

2 An island endemic is a species found on one particular island and nowhere else. There are many endemic species on the islands that make up Indonesia.

(a) Suggest why some islands have many endemic species. [5]

Many islands are surrounded by large areas of water that isolate them from the mainland or other islands and act as a barrier to movement of many organisms ✔. Any organisms that migrate to the island are likely to become isolated so that gene flow does not occur with other populations ✔. Conditions on islands are likely to be different from other places, so selection pressures are different ✔. Allele frequencies change in response to selection and allopatric speciation occur. Also the founder effect and genetic drift might occur to help form new species.

This answer has three correct ideas. The founder effect and genetic drift should be explained in the context of islands.

(b) Explain why it is important to conserve these endemic species. [3]

These species have evolved in response to the conditions on the island and the interdependence within the community depends on their specific adaptations ✔. They may have useful products that could prove useful ✔ as may their genes for improvement of crops and livestock ✔ by selective breeding or by genetic engineering ✔.

This question has been answered in two ways, both valid. One is the ecosystem approach and the other is to list the potential uses to humans of particular species.

(c) Endemic plant species are conserved in botanic gardens in Indonesia.
Suggest the difficulties that botanic gardens may have in conserving these species. [4]

The botanic gardens may not have the same conditions as in the island habitat of an endemic so it doesn't grow at all ✔. If it does grow, then over generations in the gardens it may change as a result of selection ✔ and then not fit back into the habitat if and when reintroduced. ✔

The end of the last sentence could be improved. A better answer would refer to the absence of a niche.

Exam-style questions

Structured questions (Paper 4)

1 The black-faced grassquit, *Tiaris bicolor*, is a small bird found throughout the Caribbean and the coasts of Venezuela and Colombia. It is related to Darwin's finches of the Galápagos Islands in the Pacific Ocean.

(a) Outline how variation in wing length in a population of birds, such as *T. bicolor*, would be investigated, recorded and presented. [5]

(b) Explain how it is possible to determine if the population on a Caribbean island belongs to the same species as populations on the South American mainland. [3]

(c) It is thought that a small population of birds like *T. bicolor* colonised the Galápagos Islands. Explain how such a small population may have given rise to the 14 species of finch now found on the Galápagos Islands. [7]

2 Various techniques of assisted reproduction are used in the captive breeding of rare mammals and prize livestock.

(a) Describe **two** different techniques that are involved in assisted reproduction. [6]

Three methods have been used in South Africa to control the growth of elephant populations. From the 1970s to 1994 culling was used. In response to public concerns the government banned the use of culling in 1994. Trials of contraceptives were carried out in the Kruger National Park. Implants of oestrogen were used on some female elephants and injection of a vaccine against a glycoprotein in the zona pellucida that surrounds egg cells (PZP vaccine) was tried on others.

(b) Explain why population control of large herbivores, such as elephants, is necessary in National Parks and Reserves. [3]

PZP vaccine was used to control the elephant population in the Greater Makalali Private Game Reserve from 1999. The population in 1999 was 49, and the population had been growing by 10% a year for several years before that. The table summarises changes in the population of elephants in the reserve between 1999 and 2011.

Year	Number of elephant calves born	Population size
1999	2	49
2000	5	54
2001	8	62
2002	4	66
2003	0	66
2004	3	69
2005	2	71
2006	0	71
2007	1	72
2008	1	73
2009	1	74
2010	2	76
2011	3	79

(c) (i) Explain how hormonal implants act as a contraceptive. [3]

(ii) Explain how vaccinating female elephants with PZP acts as a contraceptive. [4]

(d) Discuss the effectiveness of contraception at controlling the elephant population in the Greater Makalali Private Game Reserve. [3]

Free response questions (Paper 4)

3 (a) Discuss the roles of zoos and botanic gardens in conservation. [9]

(b) Suggest the limitations of zoos and botanic gardens in conserving endangered animals and plants. [6]

4 (a) Discuss the threats from human activities to aquatic ecosystems. [9]

(b) Explain why it is important to control the invasions of alien species. [6]

extra questions available online

19 Genetic technology

Key points

- ☐ Describe the principles of the polymerase chain reaction (PCR) to clone and amplify DNA.
- ☐ Describe and explain how gel electrophoresis is used to analyse proteins and nucleic acids, and to distinguish between the alleles of a gene.
- ☐ Outline the use of PCR and DNA testing in forensic medicine and criminal investigations.
- ☐ Define the term recombinant DNA.
- ☐ Explain that genetic engineering involves the extraction of genes from one organism, or the synthesis of genes, to place them in another organism (of the same or another species) such that the receiving organism expresses the gene product.
- ☐ Explain the roles of restriction endonucleases, reverse transcriptase and ligases in genetic engineering.
- ☐ Describe the properties of plasmids that allow them to be used in gene cloning.
- ☐ Explain why promoters and other control sequences may have to be transferred as well as the desired gene.
- ☐ Explain the use of genes for fluorescent or easily stained substances as markers in gene technology.
- ☐ Explain the advantages of producing human proteins by recombinant DNA techniques.
- ☐ Explain, in outline, how microarrays are used in the analysis of genomes and in detecting mRNA in studies of gene expression.
- ☐ Define the term bioinformatics and outline its role following the sequencing of genomes, such as those of humans and parasites, e.g. *Plasmodium*.
- ☐ Outline the advantages of screening for genetic conditions.
- ☐ Outline how genetic diseases can be treated with gene therapy and discuss the challenges in choosing appropriate vectors, such as viruses, liposomes and naked DNA.
- ☐ Discuss the social and ethical considerations of using gene testing and gene therapy in medicine.
- ☐ Explain the significance of genetic engineering in improving the quality and yield of crop plants and livestock in solving the demand for food in the world.
- ☐ Outline the way in which the production of crops may be increased by using varieties that are genetically modified for herbicide resistance and insect resistance and discuss the ethical and social implications of using genetically-modified organisms (GMOs) in food production.

Link

Make sure that you revise thoroughly the structure and function of DNA from Unit 6 before you start this Unit.

Remember

DNA is composed of two strands or **polynucleotides**. The monomers are four nucleotides, each with a purine or pyrimidine base, deoxyribose and a phosphate group.

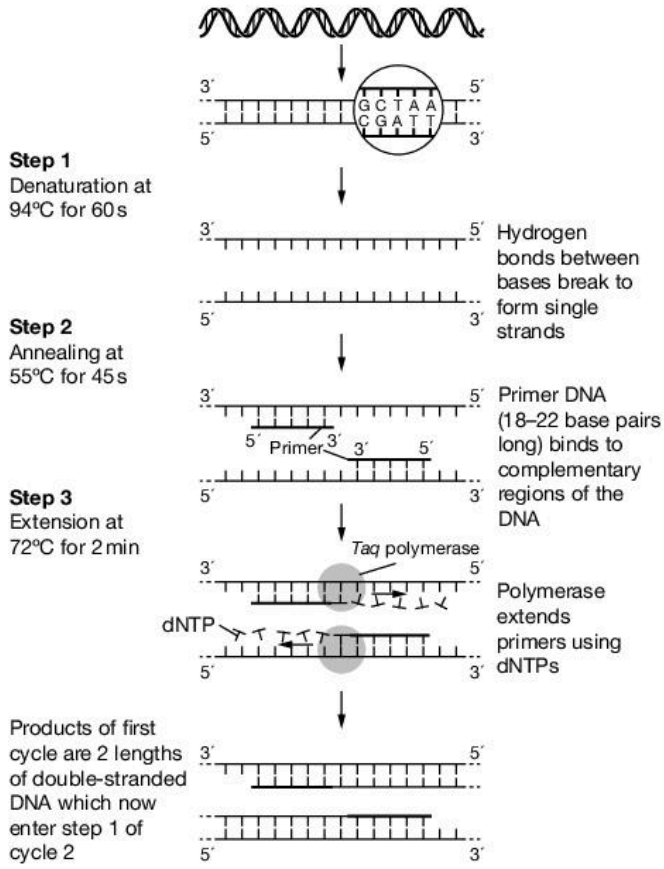
Adenine and guanine are the purine bases, thymine and cytosine are the pyrimidine bases.

Cloning and amplifying DNA

Figure 19.1 shows how small samples of DNA are **amplified** in the polymerase chain reaction (PCR).

Primers are short sequences of DNA that bind to the single-stranded DNA that is being copied at the 3′ ends. This is necessary for DNA polymerase to start the process of replicating the existing polynucleotide using **deoxyribonucleotide triphosphates** (dNTPs). *Taq* polymerase is a **thermostable** DNA polymerase that is not denatured at the high temperatures needed to separate the two polynucleotides in PCR.

Follow the steps in Figure 19.1 and notice that at the end of one cycle, a length of double-stranded DNA has been amplified to give two lengths of double-stranded DNA. After another cycle, there are 4 lengths and so the number increases exponentially as the number of cycles increases.



▲ **Figure 19.1** One cycle of the polymerase chain reaction (PCR). The reaction mixture contains the sample of DNA to be amplified, *Taq* polymerase, primer DNA and high concentrations of the four different dNTPs (dATP, dGTP, dTTP and dCTP)

Key term

Polymerase chain reaction: a technique that produces large quantities of specific DNA sequences.

Link

PCR is similar to semi-conservative replication of DNA that occurs in the S phase of the cell cycle. Read Unit 6 pages 65 and 68.

Exam tip

Search online for the Student guide for *The PCR and plant evolution* published by the National Centre for Biotechnology Education (NCBE). This gives you plenty of information about the use of PCR and electrophoresis in genome studies – in this case with chloroplast DNA (cpDNA).

Exam tip

Watch some animations that show the PCR process. See the DNA Learning Center and Max Animations for examples.

Worked example

(a) The polymerase chain reaction is used to amplify samples of DNA.

Describe the property of *Taq* polymerase that makes it more suitable for use in PCR than other DNA polymerases.

(b) State how PCR differs from DNA replication during a mitotic cell cycle.

(c) Suggest why PCR cannot increase the number of RNA molecules in the same way as it increases the number of DNA molecules.

Answers

(a) *Taq* polymerase is a thermostable enzyme. It does not denature at the temperatures (e.g. 94 °C) used to separate the two strands of each molecule of DNA in PCR.

(b) PCR occurs at temperatures between 50 and 90 °C, not at temperatures below 40 °C. The primer is a short sequence of DNA not of RNA. Replication copies the entire DNA in a cell; PCR only copies small stretches of DNA. The DNA polymerase used in PCR is thermostable; in most organisms it is not. The exceptions are the prokaryotes that live in very hot environments (extremophiles).

(c) There is no enzyme that will use an RNA template to make double-stranded RNA.

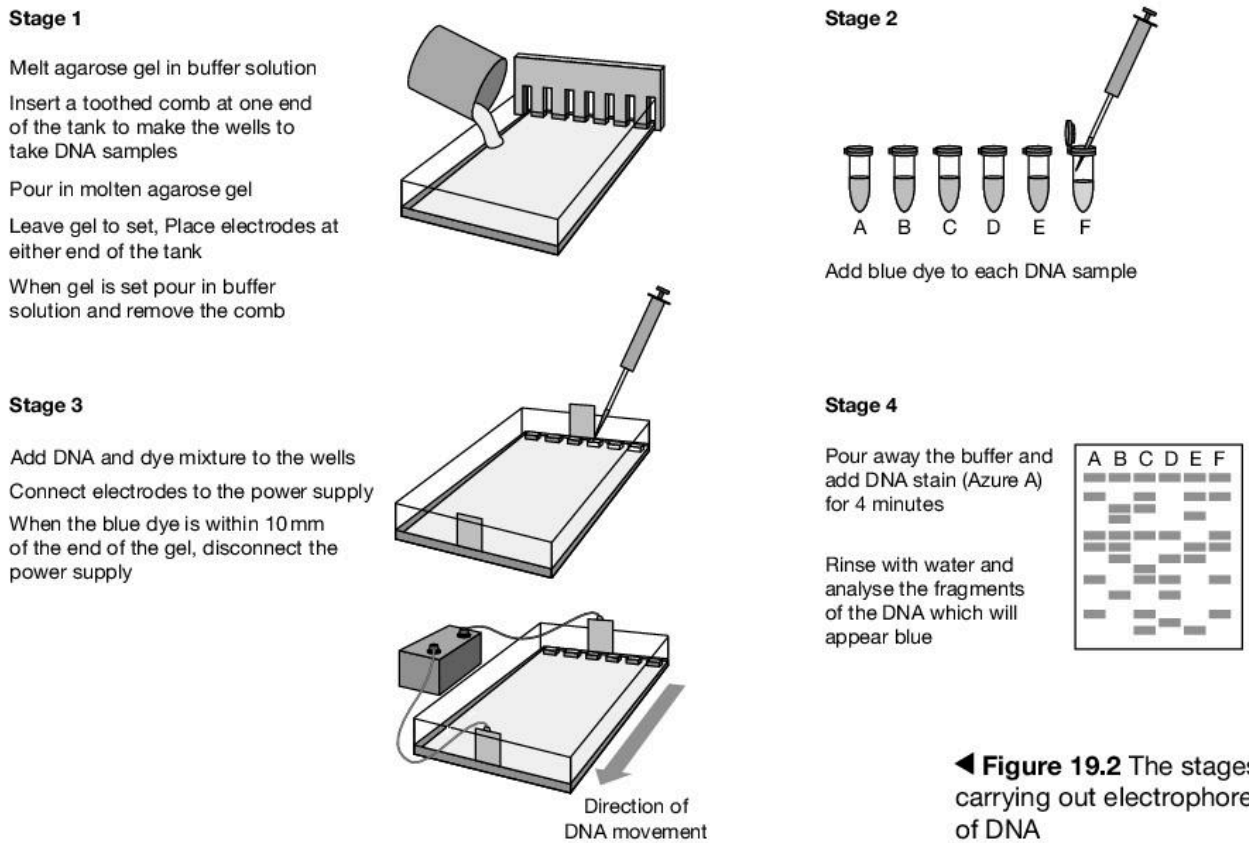
Instead, reverse transcriptase uses an RNA template to make single-stranded DNA. This DNA can then be replicated using DNA polymerase and this can be used in PCR. In this way, multiple copies of DNA can be made which hold the information in the original mRNA.

Electrophoresis

Fragments of DNA (obtained using restriction endonucleases) and proteins are separated by **electrophoresis** (Figure 19.2). Samples are put into wells cut into a gel, which is in a tank filled with a buffer solution of an appropriate pH. A direct electric current is applied to the gel and the protein molecules or fragments of DNA move towards an electrode. DNA is negatively charged so moves towards the anode. The distance moved by a fragment depends on size; smaller fragments move further per unit time than larger fragments. Proteins can be treated so that they have the same net charge and are therefore separated by mass. Alternatively, if not treated they can be separated by charge and by mass.

Key term

Electrophoresis: the separation of charged molecules by differential movement through a gel in an electric field. The degree of movement is dependent on the mass of the molecules and their net charge.



◀ **Figure 19.2** The stages in carrying out electrophoresis of DNA

DNA is invisible unless a blue or fluorescent stain is added. A radioactive DNA probe (with the isotope ^{32}P) may be used to locate specific sequences. These probes bind to complementary sequences in the DNA, making them show up as dark bands when exposed to X-ray film.

Worked example

The enzyme alcohol (ethanol) dehydrogenase is an enzyme composed of one polypeptide. There are three alleles of the gene that codes for alcohol dehydrogenase, known as *ADH1, ADH2* and *ADH3*. The three polypeptides have the same length, but different net charges and can be separated by electrophoresis.

Explain how electrophoresis can distinguish between people who are homozygous and people who are heterozygous for the *ADH* gene.

Answer

If there is a single band, then the person who provided the sample is homozygous (e.g. *ADH1 ADH1*). If there are two bands then the person is heterozygous (e.g. *ADH1 ADH2*). If a polypeptide has a net positive charge then the polypeptide will travel towards the cathode; if a net overall negative charge the polypeptide will travel to the anode. If the polypeptides are both negative or both positive, then the polypeptides will travel different distances depending on the difference in the net charge.

Uses of PCR in DNA profiling

PCR is used to produce a greater quantity of DNA from a sample of a tissue or even from an individual cell – especially from a crime scene where only trace amounts are available – to prepare a specific DNA pattern. The amplified DNA can be cut by restriction enzymes and the lengths of the fragments determined by gel electrophoresis.

Link

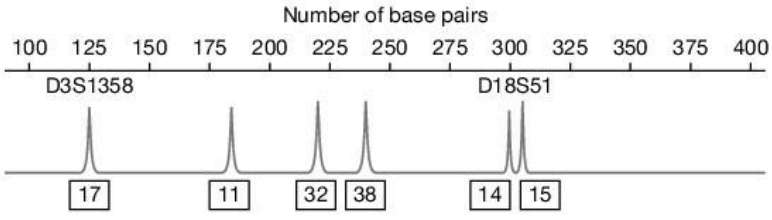
The genetic fingerprints produced by using VNTRs resemble ladders – see the patterns in Figure 19.2.

The discovery of sequences that are repeated in DNA in structural genes and in regions between them led to methods of genetic profiling (often called genetic fingerprinting). Among the first markers to be used were **variable number tandem repeats** (VNTRs), which are sequences repeated at various points along chromosomes. These are 10–100 base pairs long and are inherited from both parents. Another way to present a DNA profile uses **short tandem repeats** (STRs). STRs are regions within a gene (mainly introns) composed of 2–5 nucleotides repeated 10–30 times. The pattern shows a series of peaks that correspond to the regions of DNA that have been amplified and labelled with fluorescent dyes during the PCR process (see Figure 19.3 for part of a human DNA profile).

Originally the larger repeat sequences (VNTRs) were used but using STRs is now common and more accurate, although there are still some applications for VNTRs.

The number of repeats in any one STR is variable and is unique for an individual. The exception is identical twins, who have the same DNA profile.

In DNA profiling, different versions of a selected number of STRs are determined. Many of the most useful STRs are in regions of non-coding DNA between structural genes. DNA profiling is very useful in the forensics of criminal investigations and in missing person investigations. Each STR is given a code number and each one is like a gene with multiple alleles.



▲ **Figure 19.3** Part of a DNA profile. This shows two of the STR loci for a person's DNA profile. This person is homozygous for the locus known as D3S1358 and heterozygous for the locus D18S51. Each number refers to the numbers of repeats

Worked example

(a) DNA profiling is used in a variety of ways. Occasionally pairs of twins are separated at birth and adopted into different families so they grow up not knowing about each other.

Two people that look alike and have the same ABO and Rhesus blood groups want to know if they are identical twins.

Explain how DNA profiling provides much better evidence than blood groups that they are identical twins.

(b) Police officers find DNA at the site of a serious crime. Explain why they take samples of DNA from the victims of the crime as well as the suspected criminals.

(c) Explain why DNA profiles for individuals can show single peaks and double peaks for each STR, but never multiple peaks.

Answers

(a) There are only four different blood groups in the ABO system and two in the Rhesus system. The chances of finding two unrelated people who have the same blood group, e.g. O– or AB+, is quite high. DNA profiling uses more than the two gene loci involved in blood group antigens and all have multiple versions (multiple alleles). This makes the chance of finding two people with the same profiles extremely small, i.e. almost impossible. Only identical twins have exactly the same DNA profile.

(b) It is most likely that DNA from the victims will be collected from the scene of the crime. Their DNA will be profiled so that DNA of other people at the scene can be identified and then matched against the DNA of likely suspects.

(c) People are diploid and have two copies of each of the STRs on autosomes. A single peak indicates that the two chromosomes have identical numbers of repeats (they have the same allele). A double peak indicates that the number of repeats for the STR is different (they have two different alleles). No-one has more than two sets of chromosomes so cannot have multiple peaks.

Transferring genes

Genetic engineering is the term usually applied to the process of genetic modification by means that are not possible using traditional breeding and artificial selection. It involves the removal of a gene or genes from one organism and placing them into another. This can involve removing a gene from one species and transferring it to another species, or taking a gene from an individual of a species and transferring it into other individuals of the same species. The DNA corresponding to a gene is obtained in one of a number of ways, inserted into a **vector**, such as a virus, plasmid or liposome (small phospholipid-bound vesicles), which is used to transfer the gene into host cells.

DNA is universal, so it is possible to make transfers between widely different species – for example from a human into a bacterium, a jellyfish into a bacterium or a bacterium into a plant. The genetic code is also universal so all cells can 'read' a sequence of bases from a different species so that the protein encoded by a 'foreign' gene can be produced in any cell.

There are different ways to obtain a gene for transfer, for example:

- cut them from the DNA of the source organism
- collect the mRNA from the source organism and synthesise complementary DNA (cDNA) by using the enzyme **reverse transcriptase** (to produce ssDNA) and DNA polymerase (to produce dsDNA)
- analyse the gene product (amino acid sequence) and then synthesise the gene.

Remember

The genetic code is the sequence of three bases that code for different amino acids. It is *not* the sequence of bases in the structural genes that code for the amino acid sequences of polypeptides.

Key term

Genetic engineering: the modification of an organism's DNA, usually by the transfer of a gene from another organism, often from another species.

cDNA is complementary DNA synthesised by reverse transcriptase. ssDNA is single-stranded DNA and dsDNA is double-stranded DNA.

Restriction endonucleases are enzymes that are used to cut genes from lengths of DNA. They cut across both strands of DNA at specific sites known as **restriction sites** as they are *restricted* to cut only at these sites. The restriction sites for two of these enzymes are shown in Figure 19.4.

Many restriction sites have specific nucleotide sequences which are **palindromic** as they read the same in the 5′ to 3′ direction as in the 3′ to 5′ direction.

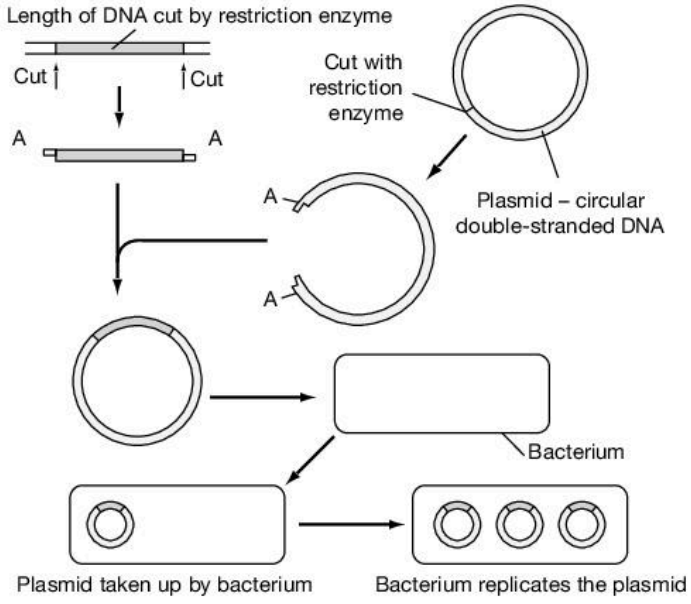
Some restriction endonucleases cut DNA to give it free, unpaired 'ends'. You can see in Figure 19.4 that *HindIII* does this. These ends are known as 'sticky ends' because they will form base pairs with complementary sequences of bases. This is how a gene cut by a restriction endonuclease can be inserted into a plasmid or into viral DNA. Others, such as *HpaI*, cut straight across DNA to give blunt ends (Figure 19.4).

Generally, the first step provides only a few copies of the desired gene (sequence of DNA) to be transferred. The sequence of DNA often needs to be cloned to produce many identical copies. This can be done by using vectors that carry the DNA into a host bacterial cell or by using PCR.

Plasmids are common vectors. They are removed from bacterial cells and cut using a restriction enzyme to produce complementary sticky ends. If the desired gene has been cut out, the same enzyme is used; if using cDNA or synthesised DNA, then sticky ends are added.

Copies of the gene are mixed with cut plasmids and **DNA ligase** (Figure 19.5). Often there are marker genes added to allow the bacteria that have taken up the gene to be identified. Also, promoter and other control sequences may be added as the bacterial cell may not have the necessary mechanism to transcribe the gene to produce mRNA (see Unit 16 page 190). Some of the plasmids will take up the gene by complementary base pairing of sticky ends (identified as A in Figure 19.5). DNA ligase seals the cut ends by forming phosphodiester bonds.

Once the gene has become inserted into the plasmid **recombinant DNA** is produced.



▲ **Figure 19.5** Plasmids are used as vectors to transfer genes into bacteria

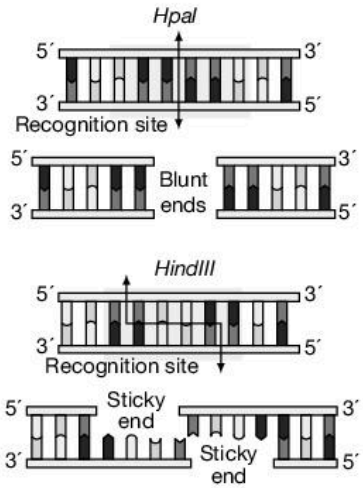
Key terms

Vector: any structure that is used to deliver a gene into a host organism in genetic engineering.

Reverse transcriptase: an enzyme that uses an RNA template to assemble a single-stranded DNA molecule (cDNA). Retroviruses have reverse transcriptase.

Restriction endonuclease: an enzyme that cuts DNA internally at specific sites. Also known as a restriction enzyme.

Restriction site: a place on DNA that has a specific sequence of bases that is recognised by a restriction endonuclease that cuts the DNA at or near the site.



▲ **Figure 19.4** The restriction sites in DNA for two restriction endonucleases

Link

Look back to the diagram of DNA on page 64 to see the hydrogen bonds between the base pairs and the position of the phosphodiester bonds in each polynucleotide.

The host bacteria are treated with calcium ions, then cooled and given a heat shock to increase the chances of plasmids passing through the cell surface membrane. Some plasmids will have resealed without taking up the gene and some bacteria do not take up plasmids. There are a variety of ways to identify the transformed bacteria from those that do not have the recombinant plasmids:

- plasmids contain genes that code for green fluorescence protein (GFP) which acts as a marker; under UV light the transformed bacteria will fluoresce
- plasmids contain the gene for the enzyme β-glucuronidase (GUS) which produces a colourless substance that is easily stained.

Gene cloning occurs in two ways in the bacteria:

- the plasmids can replicate independently to produce many copies
- the bacteria reproduce asexually to produce many recombinant bacterial plasmids.

The bacteria are described as **transgenic** or recombinant.

Bacteriophages, viruses that infect bacteria, are also used as vectors to deliver genes into bacteria. They attach to the cell wall of a bacterium and inject DNA that has been modified into the host cell. Genes which make these viruses harmful are removed from bacteriophages used as vectors.

Producing and harvesting gene products

Bacteria can make many copies of genes very quickly and can transcribe and translate the gene to make recombinant protein products. The bacteria may be grown in large quantities to make specific proteins. Bacteria do not cut out introns from transcribed DNA and cannot carry out the complex post-translation processes of eukaryotic cells to cut, fold and modify proteins by adding sugars. Genetic engineers use eukaryotic cells in culture, such as yeasts, plant cells or animal cells as the host cells for production of many proteins. In these cases control sequences that are recognised by transcription factors need to be added (see Unit 16 page 190).

The advantages of producing human proteins by recombinant DNA techniques

Genetically modified organisms (GMOs) are used to produce human proteins for use as medicines. Examples of these proteins are insulin for the treatment of diabetes, factor 8 (VIII) for the treatment of haemophilia and the enzyme adenosine deaminase (ADA) for treating severe combined immunodeficiency disease (SCID).

Production of these proteins is more reliable and is not dependent on obtaining them from animals slaughtered for the meat trade (insulin and ADA) and blood donations (factor 8). Production by GMOs removes the risk of contamination by pathogens as happened in the 1980s when blood donations were not screened properly for viruses and preparations of factor 8 were contaminated with HIV. The use of human proteins, rather than animal proteins, also reduces the risk of immune responses occurring, which would make it impossible to continue treatment.

Some medicines that are now made by recombinant DNA techniques are available in larger quantities than in the past, so costs of treatment have reduced. For example, goats have been genetically modified to produce milk containing a human protein that prevents blood clotting. Each year a GM

Link

Plasmids are small rings of double-stranded DNA found in prokaryotes. See Unit 1 page 6 and Unit 18 page 211.

Exam tip

Remember that where RNA has uracil, the cDNA has thymine. A question on reverse transcriptase might ask about this.

The advantages of using mRNA to synthesise cDNA are that there are many copies of mRNA, and RNA has no introns (that bacteria can't deal with).

Key terms

Recombinant DNA: DNA from two sources (e.g. two different species) that are joined together.

DNA ligase: an enzyme that catalyses the formation of phosphodiester bonds between the terminal nucleotides of DNA fragments to form the sugar phosphate backbone.

Transgenic organism: an organism that has DNA from another individual of the same species or from a different species as a result of genetic engineering.

goat can produce the same amount of antithrombin as can be collected from 90 000 blood donations.

Genetic engineers have changed the base sequence of the insulin gene to code for different types of human insulin. These insulin analogues act in a variety of ways. Some act faster than normal insulin (useful for taking immediately after a meal) or more slowly over a period of between 8 and 24 hours to maintain the background blood concentration of insulin. Many diabetics take both types at the same time.

Production can be easily scaled up to meet demand. Worldwide there is an increasing number of people with type 2 diabetes, many of whom may require insulin for the treatment of the latter stages of the disease.

Link

See Unit 14 for more about insulin and Table 16.3 (page 189) for more about factor 8. SCID is an immunodeficiency disease, but it is an inherited (genetic) disease unlike HIV/AIDS, which is caused by the human immunodeficiency virus (see Unit 10).

Use of microarrays

A **microarray** is based on a small piece of glass or plastic usually 2 cm^2 in size. Short lengths of single-stranded DNA probes are attached to this support with 10 000 or more different positions per cm^2. Each individual position has multiple copies of the same DNA probe. Databases hold details of DNA probes for many genes, so each type of microarray can hold probes for all the genes in an organism's genome.

Key term

Microarray: small piece of glass or plastic that has many thousands of tiny spots in known positions; each spot has many copies of ssDNA with known sequences of bases.

Microarrays are used in two ways:

- comparing the genes present in complete genomes of two different species
- finding out which genes are being expressed in tissues or cells at any given time.

Comparing genomes. DNA is collected from each species, cut up into fragments and denatured to give lengths of single-stranded DNA. The DNA is labelled with a fluorescent tag so that each species has a specific colour, for example, red or green. The labelled DNA samples are mixed together and allowed to combine (hybridise) with the probes on the microarray. Any DNA that does not bind to probes on the microarray is washed off. The microarray is inspected using ultraviolet light, which causes the tags to fluoresce. Green and red fluorescent spots indicate where DNA from one species only has hybridised with the probes. Where DNA from both species hybridise with a probe, a yellow spot is seen. Yellow spots indicate that the two species have DNA with exactly the same base sequence. This suggests that they have the same genes. No colour for a particular position on the microarray means that a particular gene is not present in either species. Microarrays are scanned and the data stored for analysis and comparison with other species.

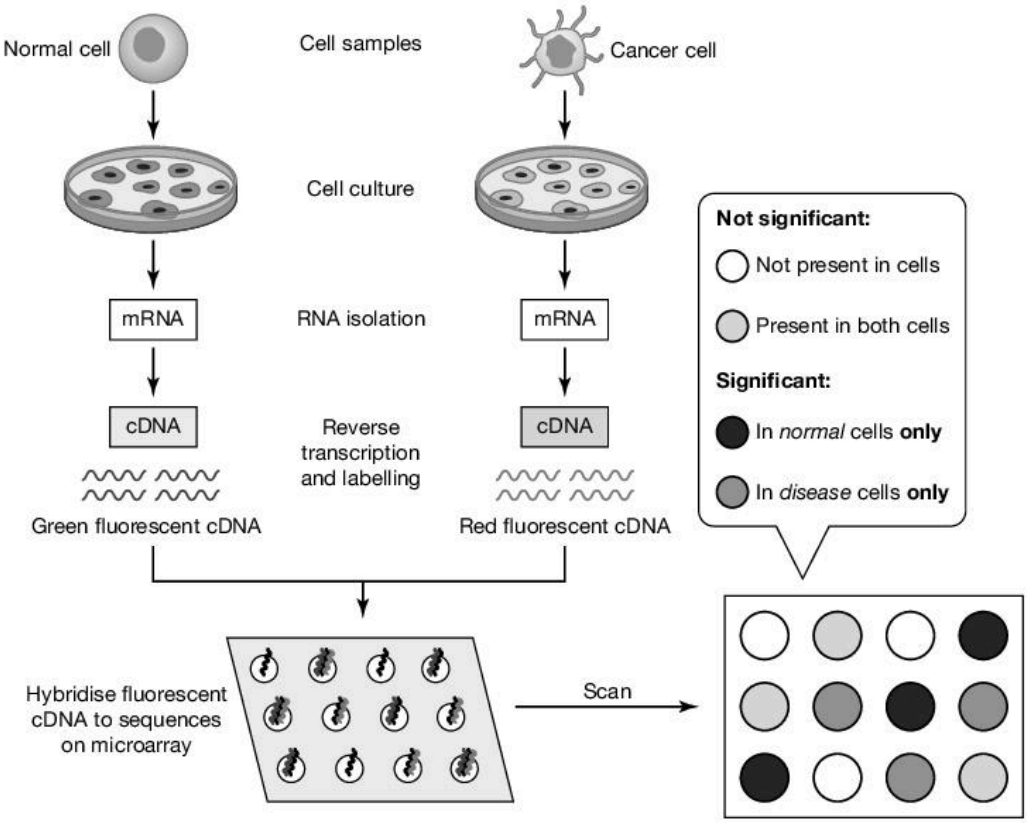
Gene expression. Microarrays are used to compare which genes are being expressed by finding those that are being transcribed into mRNA (Figure 19.6). For example, the mRNA from two types of cell is collected and reverse transcriptase is used to convert mRNA to cDNA. The cDNA is labelled with fluorescent tags, denatured to give single-stranded DNA and allowed to combine with probes on the microarray.

Exam tip

The use of microarrays in gene expression gives both qualitative and quantitative data as it indicates which genes are 'switched on' and how much mRNA is produced by transcription of each gene.

Spots on the microarray that fluoresce indicate the genes that were being transcribed in the cell. The level of activity of each gene is indicated by the intensity of light emitted by each spot. The intensity of light is proportional to the number of molecules of single-stranded cDNA that have bound to the probes. If a gene was highly active then there would be many mRNA molecules present in the sample, while a low intensity indicates that there were very few.

DNA sequencing and bioinformatics



▲ **Figure 19.6** A microarray that detects the differences in gene expression in a non-cancerous cell and in a cancer cell

DNA sequencing is the process of determining the order of base pairs in a length of DNA. Current methods of sequencing can generate base sequences of whole genomes very quickly. DNA sequencing is now a routine procedure in many aspects of biology, medicine and forensics.

Bioinformatics is the collection, processing and analysis of biological information and data using computer software. Bioinformatics allows the comparison of genomes in different organisms to investigate evolutionary relationships by looking for similarities between genes and proteins. Similar comparisons between individuals within a species are made to assess intraspecific variation at the level of base sequences. The effect of differences in gene sequences on phenotypes can also be assessed. For example, the amino acid sequences of proteins are now derived from base sequences using the genetic code (see Figure 6.5 page 66) in a very precise automated method.

Remember

Remember that the DNA in eukaryotes consists of genes that code for polypeptides, genes that code for tRNA and rRNA, control regions and other regions that appear to have no known functions.

A major focus of research once a complete genome or part of a genome is sequenced is to identify genes that code for proteins. This can be done by searching for start and stop codons and also by comparing sequences against the sequences of bases in mRNA from the organism concerned. These represent genes that are transcribed.

Detecting mRNA at different times during an organism's development gives data on gene expression that also needs to be stored and analysed. There is also a vast quantity of data about proteins: their sequences of amino acids, their shapes and functions; also their interactions with other proteins and their roles in metabolism.

There are far more prokaryote genomes that have been sequenced than eukaryote genomes.

The data is vast and growing at an exponential rate. The information needs to be in a form that can be searched and cross-matched.

All the information about the genome of *Plasmodium* is available in data bases for researchers to access. This information is being used to find new methods to control this parasite. For example, being able to read gene sequences is providing valuable information in the development of vaccines for malaria. Sequencing malarial parasites from different areas of the world shows that Cambodia is the area where mutations giving rise to drug resistance tend to arise.

Exam tip

Plasmodium is the causative organism of malaria. A question on topics in this Unit could be set in the context of information from Units 10 and 11. You are expected to use your knowledge of these Units to answer questions like this.

Human genetic diseases

Genetic screening

Genetic screening can be used to identify genes that may affect a person directly in the future (e.g. breast cancer genes, *BRCA1* and *BRAC2*; Huntington's disease) and heterozygous individuals who are carriers of genetic conditions (e.g. haemophilia, sickle cell anaemia and cystic fibrosis).

The advantages of genetic screening include:

- Information can be provided about the condition so that individuals and families can make decisions about monitoring the condition, preventative measures or any treatment that is available:
  - For haemophilia, if a woman's son has it she may want testing as she may have mild symptoms so will need monitoring during, for example, next childbirth or if she has an operation.
  - For Huntington's disease, family planning and a change of lifestyle/way of living may be advised.
  - For *BRCA1* and *BRCA2*, preventative measures could be considered.
- It may assist people with the decision on whether or not to have children. If a woman and her partner are both carriers, then they can decide to have fetal testing and, if the fetus is found to be homozygous recessive, to offer parents the opportunity to terminate the pregnancy.
- Identification of disorders in newborn babies means that treatment can be started at the earliest opportunity.

Exam tip

An advantage of genetic screening is the potential for reducing the number of people born with serious long-term, incurable conditions and thereby saving the money that would be used to pay for their treatment.

Gene therapy

Gene therapy is an application of the principles of genetic engineering. It involves the transfer of a normal functioning gene into a person who has a genetic disorder. There are a variety of methods of delivery of normal alleles into cells using:

- viruses that are taken up by specific cells
- liposomes (small phospholipid-bound vesicles)
- plasmids that can be injected directly in to cells
- naked linear DNA.

One of the major challenges in gene therapy is finding an effective delivery system that does not lead to complications. For example, trials of gene therapy for cystic fibrosis (CF) have had to be stopped because viral vectors caused inflammation. Other challenges are ensuring that the normal allele does not enter DNA where it can do harm and delivering the allele to all the cells that require it. Inserting the allele into tissue stem cells as in the treatment of SCID and β-thalassaemia (Table 19.1) is one way to ensure the normal allele enters all the cells that need it.

Exam tip

Research into gene therapy for CF has been in progress for many years. You should read information about the challenges posed in delivering the dominant allele of the *CFTR* gene into cells of the ciliated epithelium that lines the airways.

▼ **Table 19.1** Some human genetic conditions that have been treated by gene therapy in some individuals

Genetic condition	Type of gene therapy	Effect of treatment
severe combined immunodeficiency syndrome (SCID)	inserting a dominant allele for the gene *ADA* that codes for adenosine deaminase	adenosine deaminase metabolises purines in T-lymphocytes that would otherwise build up and be toxic
β-thalassaemia	inserting a dominant allele of the gene *HBB* for β-globin	restores the production of the normal form of β-globin for haemoglobin production
blindness	inserting a dominant allele of the gene *RPE65* for a pigment in photoreceptor cells	restores vision or prevents loss of vision in people with inherited forms of blindness

★ Exam tip

The conditions described in Table 19.1 are all examples of human genetic conditions that could be used as contexts for exam questions in Paper 4: SCID with topics from Unit 11, β-thalassaemia with topics from Unit 2 and receptor cells from Unit 15.

Social and ethical considerations of using gene therapy

Some of the social and ethical considerations with gene therapy are:

- The development of health problems, such as inflammation.
- Only recessive conditions, such as CF and SCID can be treated.
- Cells in the respiratory tract are short-lived and therefore any successful CF therapy that is developed in the future would have to be taken constantly.
- Many children with a genetic disease are born into families without any history of the particular disease so the parents are unlikely to have been offered genetic screening. Health authorities may consider that it is not cost effective to screen all couples for the large number of rare (or even very rare) genetic conditions.
- It is highly unlikely that gene therapy will offer any solutions to the most common disorders in humans, such as heart disease, high blood pressure, diabetes and Alzheimer's disease. These are caused by the combined effects of variations in many genes, and, thus, injecting a single gene or even a few genes may not be any good.
- People argue that we have no right to interfere with the genes that we have inherited; others reply that we have an obligation to use the knowledge that we have about human genetics and apply any successful techniques to improve and extend the life of people with these life-threatening genetic disorders.

★ Exam tip

You can read more about the issues surrounding gene therapy starting with two web sites that deal with these issues: www.beep.ac.uk and http://learn.genetics.utah.edu

Genetically modified (GM) organisms in agriculture

Improving crop plants and livestock by selective breeding has probably been done since humans began cultivating crops and domesticating animals. Traditional methods of selective breeding cannot transfer individual genes between unrelated species.

Genetic engineering gives plant and animal breeders the opportunity to select features totally new to crops and domesticated animals (see Table 19.2) and incorporate them. However, having incorporated a new gene, breeders then use selective breeding to ensure that the GM plants have all the features that farmers expect.

The first GM crop plants had genes to improve cultivation by incorporating pest and herbicide resistance. Pest resistance reduces losses to insect pests

★ Exam tip

Do not confuse selective breeding and genetic engineering. Read questions carefully to decide which of these topics is being examined. You may also be asked to compare the main features of the two methods.

like the cotton boll weevil and herbicide resistance allows farmers to spray herbicides during the growth of the crop to kill weeds that compete with the crop. A more recent development is developing crops that are designed to improve human health, for example, GM rice known as Golden rice™, which aids vitamin A production, and a GM banana, which will act as a vaccine for hepatitis B.

▼ **Table 19.2** Features of crop plants that have been improved by genetic engineering

Feature	Crop plants	Examples
disease resistance	papaya	resistance to ring spot virus
pest resistance	cotton, maize, soya	toxin (Bt toxin) coded for by a gene from *Bacillus thuringiensis* to kill pests such as boll weevil
herbicide resistance	cotton, maize, soya, tobacco	gene that gives resistance to effects of the herbicide glyphosate so allowing weed control by spraying during growth of crop
drought resistance	maize, sugar cane	genes to control water vapour loss
improved nutritional qualities	rice (as of 2017 not approved for growing by farmers other than in trials), Irish potato	several genes to produce precursors of vitamin A (vital for eye function) in endosperm of rice; increased starch content in Irish potatoes

GM animals can be divided into two groups. The first group has been genetically modified to enhance overall performance and the whole animal will become available for the food market. The second group of GM animals has been transformed to produce specific substances in milk, eggs or blood or to serve in medical research. The only animal (as of 2017) that has been given approval by a national regulatory body for consumption by humans is GM salmon in which a gene from the fish species ocean pout gives salmon the ability to grow all year round.

GM goats are an example of the second group. They make a human protein in their milk (see page 224).

Moral and ethical issues of GMOs in food production

The countries that grow most of the GM crops in the world are the USA, China, Brazil and Argentina. There has been strong opposition to GM crops in the European Union so very few are grown commercially (as of 2017).

There are several claims for the benefits of using GMOs in food production and many arguments against their use. Some of these are:

- GM technology gives plant and animal breeders the ability to introduce features that could never be introduced by selective breeding.
- The use of GM technology speeds up the changes needed to ensure crops and livestock can survive and produce high yields as environments change because of climate change, so ensuring food security.
- Farmers cannot keep seed for sowing for the following crop as GM crops do not 'breed true'; this favours large-scale commercial farmers and does not favour many farmers in developing countries.
- Genetic engineering is an expensive technology sometimes carried out by large companies whose main priorities may be profit rather than any environmental or economic consequences.
- We are dependent on seven types of grain-producing crop plants. These are all becoming genetically uniform. A loss of genetic diversity may mean that we will be unable to alter these plants if there are unforeseen future environmental challenges.

★ Exam tip

Information about GM may come from sources that are not impartial. If you read about GM crops and livestock on web sites, check which side of the GM debate they are on and treat their opinions and evidence accordingly.

★ Exam tip

Food security means having access to sufficient, nutritious food. It is a good term to use when discussing the arguments for and against GM food.

Raise your grade

1 Two problems with PCR are the high G-C composition of some fragments of DNA and the various substances present in test samples that inhibit PCR.

(a) Explain why high G-C composition of DNA should make PCR less effective. [3]

There are three hydrogen bonds between the base pair G:C compared with only two between A:T. ✔ A piece of DNA with lots of C:G pairs takes more energy to break ✔ and not all the fragments may be denatured and the strands separate so can't be copied in PCR. ✔

This answer has the right ideas. This shows how important it is in answering questions on this Unit to know the details of DNA structure.

(b) (i) Explain why *Taq* polymerase is used in PCR. [2]

Taq polymerase is not denatured by the heat used in the separation stage of PCR as it comes from a heat-loving bacterium. ✔ If a different enzyme was used it would have to be added after each stage of the process. ✔

Some more terminology (e.g. thermostable) would help this answer.

(ii) Suggest how contaminating substances might inhibit PCR. [2]

These substances might be enzyme inhibitors. ✔ The substance might enter the active site and be a competitive inhibitor of polymerase or attach to another part of the molecule and be a non-competitive inhibitor. ✔

Correct use of information from Unit 3. These substances might also combine with DNA and inhibit progress of the reaction, e.g. by binding to the primers.

(c) Describe the role of *Taq* polymerase in PCR. [4]

Taq polymerase makes copies of each strand of the DNA that is being increased in PCR. ✔ It assembles nucleotides together to make two new DNA molecules. ✔

Not enough detail for full marks. For example, *Taq* polymerase starts at the primer on both strands and adds the nucleotides in sequence until it reaches the end of the chain.

(d) PCR can make genes for genetic engineering. Some polymerases supplied for PCR also have exonuclease activity to produce an overhang consisting of several adenine bases. Suggest the advantage of the exonuclease activity of some polymerases. [2]

This produces sticky ends with unpaired adenine bases ✔ that can attach to a complementary sequence of thymine bases e.g. on a plasmid. ✔ Correct answer.

(e) Outline how PCR is used in DNA profiling. [3]

PCR amplifies the DNA found at crime scenes. The primers used in PCR are for several regions that are used as 'markers'. ✔ The DNA is taken from any suspects or other people who may have left their DNA at the crime scene and that is amplified in the same way. Any identical match means that there is a very high probability that the person was at the scene of crime and may have committed the crime.

The candidate needs to mention that the primers used are specific to certain base sequences that have tandem repeats. PCR indicates the number of repeats and these can be used to match samples taken from suspects to the sample taken from a crime scene.

2 (a) The following are used in genetic engineering:

A an enzyme that synthesises new DNA

B enzymes that cut DNA at specific sequences

C an enzyme that forms phosphodiester bonds to attach fragments of DNA together

D small, circular pieces of DNA found in bacteria that act as vectors

E an enzyme that uses an RNA template to make DNA.

Name **A** to **E**. [5]

A polymerase ✘ **B** endonucleases ✘ **C** phosphodiesterase ✘ **D** liposomes ✘ **E** RNA polymerase ✘

This should be an easy question to answer as it simply requires knowledge of the terms used to carry out genetic engineering. The candidate should have revised the terms listed in the syllabus and learnt what they mean.

(b) Promoter sequences fulfil important roles in cells.

State the role of promoters in genetically-modified cells. [2]

Promoter sequences are attachment sites for RNA polymerases and transcription factors. ✔ The sequences are 'upstream' of each gene. Most genes inserted by genetic engineering must have a promoter sequence to allow it to be transcribed in the host cell. ✔

This is a full answer with good use of terminology. Note the reference to transcription factors from Unit 16.

(c) Complex human proteins, such as antithrombin, cannot be produced by genetically modified bacteria. Suggest why bacteria are unable to produce complex human proteins such as antithrombin. [3]

Bacteria do not process polypeptides after translation by adding sugars to produce glycoproteins. ✔ Also they do not secrete them so have to be broken open. ✔

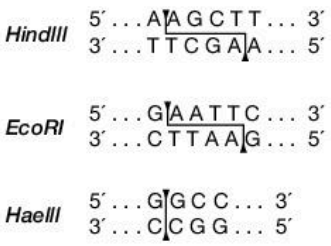
These are two good reasons.

Exam-style questions

Structured questions (Paper 4)

1 **(a)** Three restriction enzymes are *HindIII*, *EcoRI* and *HaeIII*.

The diagram shows the restriction sites for these three enzymes.



(i) Describe the features of the restriction sites shown in the diagram. [3]

Three identical lengths of DNA were treated *separately* with each of the restriction enzymes. The DNA has the following sequence of base pairs:

5′ AGTTGAAAGGCCTTCATCGCACCCTTAATTCGTGGCCAAGCTT 3′

3′ TCAACTTTCCGGAAGTAGCGTGGGAATTAAGCACCGGTTCGAA 5′

(ii) Use the information about these restriction endonucleases to state how many fragments of DNA will be present after treatment with each of the restriction enzymes. [3]

(iii) Explain why some of the fragments need further treatment before they can be inserted into plasmid vectors. [2]

(b) RNA can be incorporated into the genome of retroviruses to use as a vector for the genetic modification of animal cells. Suggest and explain how this approach to genetic engineering can lead to the production of transgenic animal cells. [3]

2 Several species of crop plant have been genetically engineered to express the gene *cry* from the bacterium, *Bacillus thuringiensis* (Bt). The gene codes for a protein that is toxic to some insect pests. GM varieties of soya, oil seed rape, cotton and maize are grown in countries such as the United States, China, Canada and Brazil.

(a) Explain the advantages of growing varieties of these crop plants with the *cry* gene for the Bt toxin. [4]

(b) Outline how crop plants, such as those listed above, are genetically modified to improve productivity. [4]

(c) Outline the potential risks of growing GM crop varieties and suggest steps that can be taken to minimise them. [5]

Free response questions (Paper 4)

3 **(a)** Explain, in outline, how gene therapy is carried out. [7]

(b) Discuss the disadvantages and the potential advantages of gene therapy. [8]

4 **(a)** Outline how the polymerase chain reaction (PCR) is used to produce 16 copies of the DNA from a single cell discovered at the scene of a crime. [8]

(b) Researchers working on the genomes of giraffes have shown that there are four distinct species of this mammal in Africa instead of one.

Explain how researchers investigate genomes to show the presence of different species. [7]

extra questions available online

20 Practical assessment

Paper 3 (40 marks)

Paper 3 tests your ability to carry out practical work and is taken in a laboratory at your school or college or in a special centre (e.g. at a university). There are usually two, or sometimes three, questions: one question is usually an investigation in which you follow instructions and make decisions about the method and the recording and interpretation of results; a further question usually involves using a microscope and tests your drawing skills.

Paper 3 is set on Units 1 to 11 only

Marks are awarded for the following skills:

Manipulation of apparatus, measurement and observation (MMO)	Marks
Making decisions about measurements or observations	8
Successfully collecting data and observations	8
Presentation of data and observations (PDO)	
Recording data and observations	4
Displaying calculations and reasoning	2
Data or observations layout	6
Analysis (interpretation of data or observations), conclusions and evaluation (ACE)	
Interpreting data or observations and identifying sources of error	6
Making conclusions	3
Suggesting improvements to a procedure or modifications to extend an investigation	3

Maths skills, such as calculating percentages, percentage changes, means and magnifications are also assessed.

Manipulation of apparatus, measurement and observation (MMO)

In Paper 3, you follow instructions and make decisions. You are instructed to read through the whole paper before starting any practical work. Read through each question and make notes on the paper. Highlight or underline the information given about the topics from the syllabus that provide the context for each question.

Working with solutions

In Paper 3, you may have to make up solutions from a stock solution. In Paper 5, you should also know how to make the stock solutions.

To make a stock solution of 100 cm^3 of 10 g 100 cm^{-3} (a 10 % solution) of glucose:

- use a balance to weigh 10 g of glucose
- dissolve the glucose in about 50 cm^3 warm water
- pour the solution into a measuring cylinder or volumetric flask and add water to the 100 cm^3 level (use a dropping pipette to add the last few cm^3 of water)
- pour into a labelled beaker and stir thoroughly.

Exam tip

Use this reading time to recall relevant aspects of your subject knowledge and to write down anything useful in the margin or on any white space.

Remember

For a solution, use *volume* and *concentration* and avoid using 'amount', which can be used for concentration, volume or number.

Link

You can find examples of simple (proportional) and serial dilutions in Units 2, 4 and 15.

To make a stock solution of 100 cm^3 of 1 mol dm^{-3} sucrose:

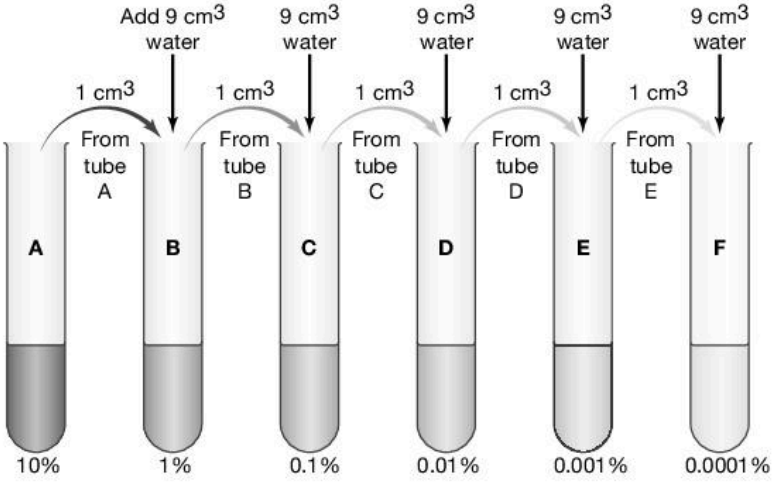
- look up the relative molecular mass (RMM) of sucrose = 342 g mol^{-1} (3 s.f.)
- use a balance to weigh 34.2 g of sucrose (a tenth of the RMM)
- dissolve the sucrose in about 50 cm^3 water
- pour the solution into a measuring cylinder or volumetric flask and proceed as above.

You should be able to describe how to make different percentage and molar concentrations using stock solutions and water.

You can make a range of concentrations from the 10 g 100 cm^{-3} stock solution of glucose. For a wide range of concentrations, use **serial dilution**. For a narrower range with smaller intervals between the extremes of the range, use **simple (proportional) dilution**.

Serial dilution

1. Use a graduated pipette or syringe to remove 1 cm^3 of the stock glucose solution. Put it into a test tube labelled with the appropriate concentration.
2. Add 9 cm^3 of water and stir or invert the test tube to mix. This is now a 1.0 g 100 cm^{-3} solution (1%).
3. Repeat this (see Figure 20.1) until you have a 0.0001 g 100 cm^{-3} solution.



◀ **Figure 20.1** Making a serial dilution – each transfer reduces the concentration of glucose by a factor of 10

Simple (proportional) dilution

▼ **Table 20.1** Making glucose solutions by simple dilution. The volumes of a 10 g 100 cm^{-3} (10%) stock solution of glucose and distilled water used to make four dilutions

Concentration of glucose / g 100 cm^{-3}	Volume of 10 g 100 cm^{-3} glucose solution / cm^3	Volume of distilled water / cm^3
0	0	10
2	2	8
4	4	6
6	6	4
8	8	2
10	10	0

Exam tip

You may be asked to make percentage solutions. Remember that a 10 g 100 cm^{-3} solution is also known as a 10% solution. This is the same concentration as a 1.0 g dm^{-3} solution.

Exam tip

Always read the level of a solution by looking at the *bottom* of the meniscus. Avoid parallax error by having your eye on the same level as the meniscus.

Exam tip

If asked to find the lowest concentration of reducing sugar that can be detected with the Benedict's test, use a serial dilution and test each concentration in *exactly the same way*. This will find the **sensitivity** of the test.

Exam tip

You can make up serial dilutions using other factors, e.g. by a factor of 2 by transferring 5 cm^3 each time to make a maximum volume of 10 cm^3. Starting with 10% stock solution, this would give 10%, 5%, 2.5%,1.25%, 0.625% and 0.3125%.

1. Use a syringe or graduated pipette to put certain volumes of the stock solution into test tubes, each labelled with the appropriate concentration.
2. Add sufficient water to each test tube to make the same *total* volume in each (Table 20.1).

To calculate the volume of stock solution you need in column 2, use this formula:

$$\text{volume of stock solution required} = \frac{\text{concentration wanted} \times \text{volume wanted}}{\text{concentration of stock solution}}$$

Investigations of enzyme activity often involve making different concentrations of an enzyme. Some enzymes are supplied as a liquid, not a powder. To make 100 cm^3 of a 1% solution, add 99 cm^3 of water to 1 cm^3 of the liquid enzyme.

★ Exam tip

In simple dilution, it is best to increase the concentration using even intervals as in Table 20.1.

Standardising variables

Standardised or controlled variables are variables that are kept constant to ensure that the results of the investigation are valid (see page 241).

Temperature is best controlled with a water bath. You will be provided with a thermometer and beaker(s) of water near to the temperature(s) you need to use. You may also be supplied with a means to heat the water. Follow these tips:

- stir the water with the thermometer before taking a reading
- put the bulb of the thermometer in the middle of the container of water when you take the reading
- keep checking the temperature to make sure it does not fluctuate too much
- record the temperatures on your exam paper as evidence of your attempt to keep the temperature constant.

pH can be controlled by using a buffer solution. You will be given pH papers (not pH meters) to monitor the pH of your reaction mixtures, so keep a record of the pH. Sometimes a *change* in pH is the dependent variable, e.g. the breakdown of triglycerides to fatty acids by lipase; breakdown of urea to ammonia by urease.

★ Exam tip

The range in the serial dilution shown in Figure 20.1 is 0.0001 g 100 cm^{-3} to 10 g 100 cm^{-3}, which is an increase of a factor of 10^5. In the simple dilution in Table 20.1, the range is 2 g 100 cm^{-3} to 10 g 100 cm^{-3}, which is an increase of a factor of 5.

★ Exam tip

Humidity and air speed may need to be kept constant when using potometers, which are described in Unit 7 page 81.

Uncertainty in measuring

The scales on apparatus used for measuring linear dimensions and volumes are divided into sections. The **resolution** of each piece of apparatus is the smallest division on the scale: for a typical ruler it is 1 mm; for plastic syringes it varies, but for the one in Figure 20.2, it is 0.2 cm^3.

Key term

Resolution: the smallest division on the scale of a piece of apparatus, e.g. a ruler or a plastic syringe. The smallest measurement possible with any digital apparatus.

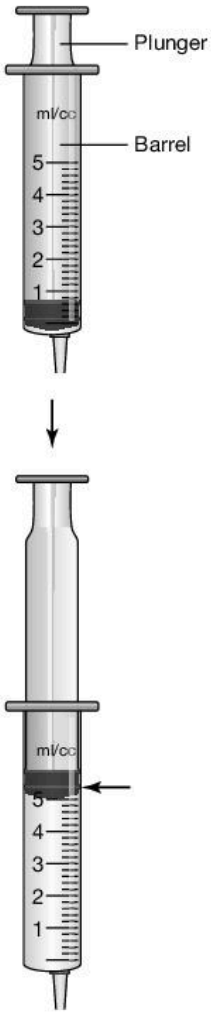
The **uncertainty of measurement** is half the smallest graduation on the apparatus. For example, for a plastic ruler with a smallest division of 1 mm, the uncertainty is ±0.5 and this applies to both where you align on zero and where you take a measurement. If a drawing measures 50 mm long, you can be certain that it is more than 49 mm but less than 51 mm, so express the length as 50 ± 1.0 mm.

★ Exam tip

A student calibrates an eyepiece graticule at a magnification of ×400 (high power). Each small division on the graticule scale = 2.5 µm, so the uncertainty is ±1.25 µm. The width of a cell is measured as 45 µm ± 2.5 µm, as uncertainty applies at both sides of the cell where the scale is placed. The percentage error is 5.6% (2 s.f.).

★ Exam tip

When measuring with an eyepiece graticule, the uncertainty is half the smallest division on the graticule, whatever that is when you calibrate it (see Unit 1). If you do not calibrate the graticule, the uncertainty is 0.5 EPU (eyepiece unit).



▶ **Figure 20.2** To dispense 5 cm³ of a liquid with this syringe, push the plunger to the bottom of the barrel. Put the nozzle into the liquid and raise and lower the plunger several times to make sure there are no air bubbles. Pull out the plunger slightly above the 5 cm³ mark. Remove the syringe from the liquid and hold it at eye level. Push in the plunger till the part shown by the arrow is over the 5 mark. Then push the plunger right to the bottom of the barrel to expel 5 cm³ of liquid

When using a syringe to measure volumes, the smallest division for a 5 cm³ syringe (Figure 20.2) is 0.2 cm³. If the syringe is used to take up and deliver 5 cm³ water, the volume should be recorded as 5.0 ± 0.1 cm³ as you are only taking a reading from the scale at one place and do not have to look at the scale for zero. If, however, you are delivering 2 cm³ of a liquid with the same syringe by moving the plunger from 5.0 cm³ to 3.0 cm³, then you are reading from the scale at *two* places and the volume should be recorded as 2.0 ± 0.2 cm³.

The uncertainty on any digital apparatus is the resolution of the apparatus in each measurement. For example, the uncertainty of a balance that reads to 0.01 g is ±0.01 g for each measurement you take.

You can calculate the **percentage error** for apparatus used for measuring, both when setting up your experiment and when taking results. For example, 5 cm³ of gas collected and measured with a gas syringe that has graduations every 1 cm³ contains more than 4.5 cm³ but less than 5.5 cm³. Your error is ±0.5 cm³ in 5 cm³. This gives:

$$\text{percentage error} = \frac{0.5}{5.0} \times 100 = 10\%$$

If 10 cm³ of gas is collected, the percentage error is 5%.

Presentation of data and observations (PDO)

Processing data

Readings you record from your investigation are sometimes known as 'raw data'. You may have to process the data by calculating a percentage, a percentage change or a rate.

Calculating a percentage: Percentages are a way of making valid comparisons between items when the totals are different. For example, 20 in 200 cells near the root tip of garlic divide by mitosis (10%), but further up the root only 1 in 50 are dividing (2%). The percentage is the value divided by the total multiplied by 100.

Calculating a percentage change: Percentage change is a valid way to compare the change to the original value at the start. The percentage change is calculated as:

$$\text{percentage change} = \frac{\text{difference between original and final numbers}}{\text{original number}} \times 100$$

Calculating rates of reaction: If you collected a gas, then rates of reaction can be calculated as the volume collected per unit of time (e.g. seconds (s) or minutes (min)):

$$\text{Rate of reaction} = \frac{\text{volume of gas collected over a period of time}}{\text{time taken}}$$

The units will be $\text{cm}^3\,\text{s}^{-1}$ (cm³ per second) or $\text{cm}^3\,\text{min}^{-1}$.

If you recorded the time taken to reach an end point (t), calculate the reciprocal of time taken $\left(\frac{1}{t}\right)$ and multiply by 10, 100 or 1000 to give a range of numbers greater than 1.

★ **Exam tip**

If the percentage error is large, increase the size of the measurements in your investigation to *reduce* the error as a proportion of the measurements that you take.

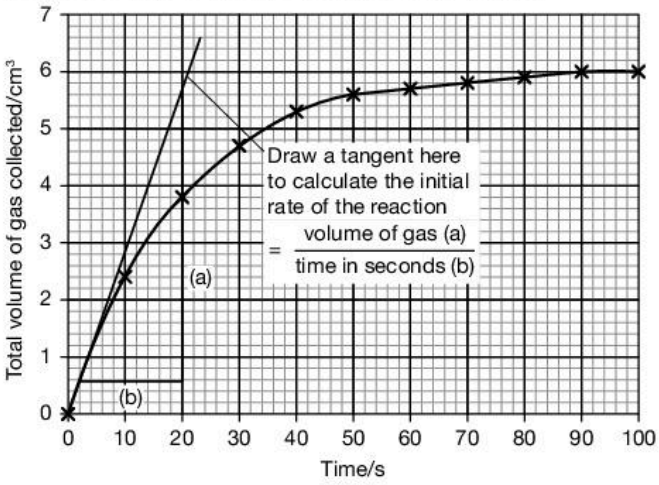
Key term

Percentage error: uncertainty in measurement expressed as a percentage of the total measurement.

★ **Exam tip**

If the percentage change is an increase, put a plus sign (+) in front of the answer; if it is a decrease put a minus sign (–).

Calculating initial rates of reaction: Enzyme activity is often measured by determining the **initial rate** when substrate concentration is not limiting. You can find this by taking measurements over a short period of time, plotting them on a graph and using a tangent to calculate the rate, as shown in Figure 20.3. Use points to calculate the rate that are separated by at least half the length of the tangent you have drawn.



▲ **Figure 20.3** Calculating the initial rate of an enzyme-catalysed reaction

Significant figures

Express the results of any calculations to the same number of significant figures (s.f.) as the least accurate figure used, which will have the lowest number of significant figures. If your input data has 3 s.f., your calculated answer should not have more than 3 s.f. If the numerator has 4 s.f. but the denominator has 2 s.f., your answer should only have 2 s.f.

If your calculation has more than one step, do not round up the number until after the last step in your calculation.

Recording results in tables

You will usually need to record the results of your practical work in tables:

- use the space provided and do not start right at the top of a page; draw table outlines (columns and rows) with a sharp pencil
- make the first column the **independent variable** and second and subsequent columns the **dependent variable**(s)
- write brief, but informative headings for each column and put units in the headings *not* in the body of the table
- use the appropriate SI unit for each column headed with a physical quantity, e.g. g for mass; cm^3 for volume
- use a solidus (/) or brackets to separate the physical quantity from the unit in which it is measured, e.g. distance / m or distance (m); be consistent all the way through your answers
- use 'per' or the negative exponent, e.g. cm^{-3}, in units; do *not* use a solidus to mean 'per', e.g. for concentration use g per dm^3 or $g\,dm^{-3}$ *not* g/dm^3
- organise data so that patterns can be seen – arrange the values of the independent variable in ascending order, i.e. values increase down the table
- make the body of the table brief – single words, short descriptive phrases, numbers, ticks or crosses, etc.

★ **Exam tip**

Devising a scale on a graph with figures that are much less than one can be difficult. That's why it is a good idea to multiply rates of reaction calculated as $\frac{1}{t}$ by 10^1, 10^2, 10^3, etc.

This graph shows the increase in the product over time. The concentration of the product eventually remains constant because there is no more substrate left.

★ **Exam tip**

On the Exam paper, plan your table so that there is space left around the sides and at the bottom in case you need to enter more columns or rows.

★ **Exam tip**

You can put the numbers/letters used for labelling in a column to the left of the independent variable.

★ **Exam tip**

The numerator is the number above the line in a calculation; the denominator is the number below the line.

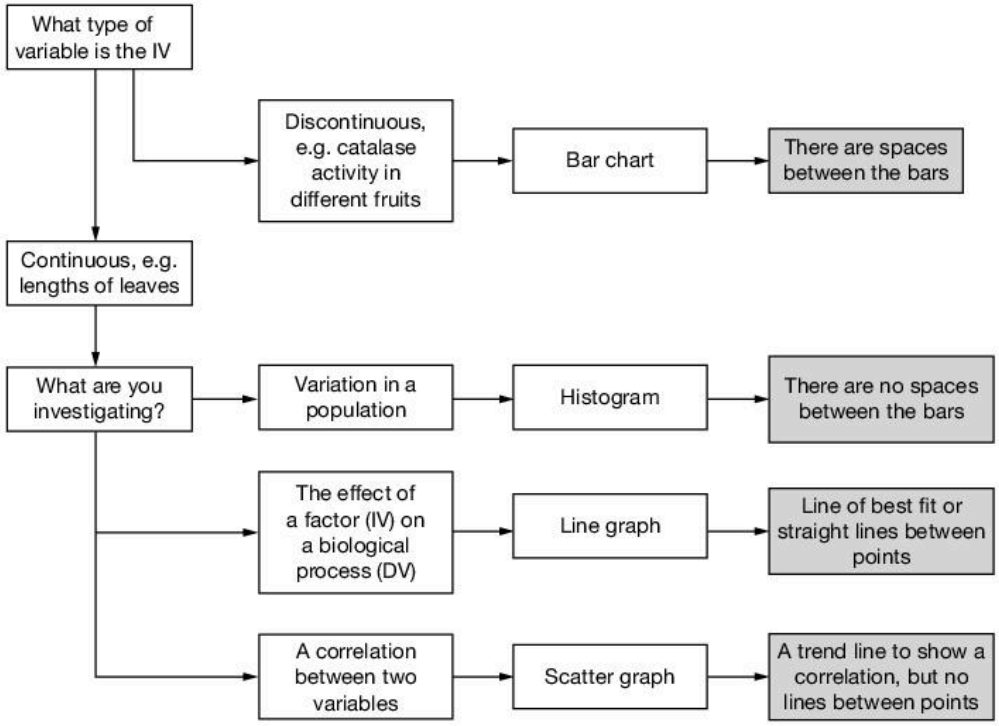
Key terms

Independent variable (IV): factor that is changed by the experimenter. Values of the independent variable are known at the start of the experiment.

Dependent variable (DV): factor that is measured by the experimenter. Values of the dependent variable can be predicted but are unknown at the start of an experiment.

Presentation of data

Data can be presented as pie charts, bar charts, histograms, line graphs and scatter graphs. Use Figure 20.4 to help you decide the most appropriate way to present your data.



▲ **Figure 20.4** How to choose the correct way to present data. The shaded boxes show the key points about each graph or chart

Bar charts: Follow this guidance for drawing bar charts:

- use most of the grid provided – do not make the bar chart too small
- draw with a sharp pencil
- use lines, or more usually, blocks of equal width separated by spaces
- make the spaces between the bars on the *x*-axis equal
- scale the *y*-axis with equidistant intervals, usually starting at zero written at the base of the axis
- label the *y*-axis with the heading and unit taken from the table of results
- arrange the bars in the same order as in a table of results
- clearly identify each bar
- do not shade the blocks as it uses up valuable time.

Histograms: Follow the general guidance for bar charts and also:

- scale the *x*-axis with equidistant intervals as it represents continuous data; write these underneath, e.g. '3.0–3.9' means that 3.0 is included in this class, but 4.0 is not; 4.0 will be included in the next class: 4.0–4.9
- label the *x*-axis and use the appropriate unit
- scale the *y*-axis properly; it can show percentage frequency; start the scale at zero written at the base of the axis

Link

See Figure 17.1 on page 195 for an example of a bar chart.

Link

Bar charts can be used to show mean results with error bars. Standard deviations, standard errors or 95% confidence intervals can be used as error bars. See the section on descriptive statistics on the support website for further information.

Link

See Figure 17.2 on page 195 for an example of a histogram.

- label the *y*-axis as appropriate, e.g. 'number' or 'percentage frequency'
- draw the blocks touching
- make the widths of the blocks equal as the *area* of each block is proportional to the size of the class and it is usual to have similar-sized classes.

Line graphs: The term *graph* applies to the whole representation and not just to the line. Line graphs are used to show relationships in data that are not immediately apparent from tables. The lines on graphs are straight trend lines, curved trend lines or straight lines between plotted points (dot-to-dot).

- Use at least half the grid provided, do not make the graph too small.
- Plot the independent variable on the *x*-axis.
- Plot the dependent variable on the *y*-axis; sometimes this is a **derived variable**, for example, rates or percentages (see Figures 3.5 and 4.6).
- Use an appropriate scale for each axis; examine the data critically to decide whether to start the scale(s) at zero; if not, one or both axes may have a **displaced origin** (see Question 2 page 160 and Question 3 page 247).
- Label each axis clearly with the quantity and SI unit or compound units as appropriate, e.g. time/s and concentration/g dm^{-3}.
- Make the plotted points easily distinguishable from the grid lines; use encircled dots (⊙) or saltire crosses (x), *not* dots on their own. If you need to plot three lines, vertical crosses (+) can also be used. Plot all points on the grid, *not* on the white space around the grid.

After plotting the points, decide if there are any **anomalous results** (outliers). Ask 'Do they fit the trend?'. Knowledge of the theory behind the investigation, will mean you are aware of the likely trend. Circle any results you think are anomalous. Make a key outside the graph to show that circled point(s) represent anomalous result(s). Then decide how to present the line.

- The points may lie on an obvious straight line. Decide whether to include the origin (0, 0) if it is not a datum point in your results. If it is, then place a clear plastic ruler on the grid and draw a straight line from the origin to give an even number of points on both sides of the line. If the origin is not a point or you are unsure, start the line at or near to the first plotted point. Do *not* continue the line past the last plotted point.
- Draw a smooth curve only if you know that the intermediate values fall on the curve. When you expect the relationship to be a curve and the points seem to fit on one, then draw it. Decide whether the origin is a point and, if not, start at the first plotted point. The curve should go through as many points as possible, but make sure there is an even number of points on either side of the curve. Do not continue past the last plotted point.
- When you are unsure if the relationship is a straight line or a curve, draw straight lines between the points. This indicates uncertainty about the results for values of the independent variable between those plotted.
- When a graph shows more than one line or curve, label the curves or use a key to show what each line represents.

Exam tip

There is no need to give a graph or a table a title on the exam paper as this wastes time and does not gain any marks.

Exam tip

For ease of plotting, use 1, 2, 5 or 10 or multiples of these for scaling axes, not 3 or multiples of 3. If your scale is 20 mm = 3 cm^3, each small square on the graph paper = 0.3 cm^3, which makes plotting points and interpolating (taking figures) from a line graph difficult.

Link

See Unit 17 page 195 if you are unsure what is meant by the term 'class'.

Link

Line graphs are often used for extracting data. See Question 1 on page 36 and Question 5(b) on page 102 for examples.

Key terms

Derived variable: variable produced by processing the data collected in an investigation.

Anomalous result: any result that does not agree with the trend of results (i.e. for any value of the independent variable) or any replicate result that is significantly different from most of the others.

Link

For examples of tables and graphs with derived variables, see Figures 3.9, 12.16, 13.12 and Tables 4.4, 12.9, 13.3.

Exam tip

When there is no data for (0, 0), but the origin is obviously a point, include it.

Analysis (interpretation of data or observations), conclusions and evaluation (ACE)

Accurate data

In an investigation, you may be asked to obtain values from samples prepared for the exam. For example, you may use the Benedict's test or the biuret test to estimate the concentration of reducing sugars or proteins in solutions given to you. The examiner and the technician who made up the samples will know these values. Your results can be compared with the true values to check the **accuracy** of your results.

During your investigation, you may not have time to take two or more results for each value of the independent variable – **replicate results** (replicates). **Repeatable results** are replicate results that are in close agreement. You can describe the variation in replicate results using maths (see the support website).

Reproducible results are results obtained by someone else who has followed exactly the same procedure as you. In an exam, you can only comment on reproducibility if you are given some results to compare with yours.

When you carry out an experimental procedure, evaluate the quality of the data collected and the way in which you carried it out. Ask yourself 'Can I have confidence in my procedure and in my data?' If you do not have confidence in the data then you cannot have confidence in the conclusion(s) that you make. Questions are often asked in these terms.

Errors in measurement

You may have to identify any errors in your investigation:

Systematic errors are usually due to some fault with the measuring apparatus and have the same effect across the full range of measurements throughout an experiment. For example, a measuring device that is always wrong by a certain value, or a controlled variable that is always incorrect by the same quantity. When there are small systematic errors (that are always the same), the data may be precise but not accurate.

Random errors are caused by unknown and unpredictable changes in the experiment; the procedure is not exactly the same each time or the apparatus is read in a slightly differently way each time you take a reading. These errors often affect some results, but not all of them, and they do not always affect the results in the same way. Random errors can also be due to variation in biological material. These are *not* mistakes or due to poor technique. Their effect is reduced by replicating experiments and calculating mean results.

Evaluation

Identify the possible limitations of your method. Highlight on the question paper any aspects of the procedure that you found difficult or that needed extra care. For example, early results may be inaccurate if you were not confident at the start and didn't have time to repeat them. Also, timing may be a problem as you cannot start a stopwatch or bench timer at exactly the same time that you start another procedure. If you are always too slow starting the timer, you have a systematic error. If your timing method improved during the investigation, you have a random error that is more significant for the early readings.

Consider the effect of the limitations you have identified on the quality of your data. For example, starting your timer too early will make the time recorded too long and overestimate the time. Starting your timer too late will

Exam tip

You need to be able to use, analyse and interpret graphs that you draw and those given in any of the exam papers, not just those in Paper 3.

Exam tip

Take care using the term accuracy. Very few biology investigations can determine true value(s) exactly; for example, no-one will know the exact rates of reaction in an investigation into the effect of pH on the activity of catalase.

Key terms

Accuracy: closeness between any measured value and the true value.

Replicate results: repeated results for the same value of the independent variable.

Exam tip

Terms and phrases, such as **uncertainty** and **accuracy**, are used when discussing experimental work. Make a list of these with their definitions and the contexts in which they are used.

make the time recorded too short and underestimate the time. When you calculate rates of reaction, a longer time underestimates the rate and a shorter time overestimates the rate.

You can also expect to suggest an improvement for each limitation. Make suggestions for the investigation you have carried out. Do not plan a new investigation or devise a totally new procedure. For example, investigating the effect of pH on enzyme activity may not produce valid results if you did not use a thermostatically-controlled water bath (limitation) as the temperature of the laboratory may fluctuate (temperature not controlled) and enzyme activity increases when the temperature increases (explanation). You could suggest repeating the investigation, this time using a thermostatically-controlled water bath set to the same temperature for all replicates (modification/improvement).

Making conclusions

There are three marks available in Paper 3 for using your knowledge to explain the results that you have obtained. As you practise past paper questions, learn to identify these questions and think about them as you carry out the practical work.

Validity refers to the confidence that you can have in your data and your conclusions. If you have a valid investigation then you have measured what your experiment was designed to measure. If asked about the validity of an investigation, consider the following:

- the limitations in the procedure
- any uncontrolled variables
- the effects of errors (systematic and random) on the results
- the repeatability of the results
- the **precision** of the data collected
- the accuracy of the results.

Key terms

Validity: the idea that an experiment really investigates the hypothesis that is being tested and whether the results really indicate what an experiment was designed to measure.

Precision: closeness of repeated measurements (replicates) to each other.

★ Exam tip

Before you write your conclusion, look back to the beginning of the question to remind yourself of the topic from the syllabus that provides the context for the question.

Microscopy

In the exam you may have to share a light microscope with another student. You may have to start with the microscopy question so the other student can use it for the second half of the exam. In the microscopy question, the examiners may ask you to:

- make a temporary preparation of a plant tissue (e.g. onion epidermis, see page 7) or some cells (e.g. yeast)
- use a microscope slide that has been prepared specially for the exam
- use photomicrographs or electron micrographs that are printed on the exam paper.

Temporary preparations of plant tissues may involve smearing material on a slide, cutting sections of stem, root or leaf, dissecting out plant tissues, pulling tissues from organs or breaking up tissues with a needle (macerating).

These are typical instructions to make a temporary preparation of the lower epidermis of a leaf.

1. Tear the leaf across and use your fingers or a pair of forceps to remove a piece of the lower epidermis.
2. Place the piece of epidermis with the external surface facing upwards in a drop of water on a slide. Use a mounted needle to gently lower a coverslip to cover the tissue.

★ Exam tip

Practise making temporary preparations and viewing them with a microscope. This will improve your manipulative skills.

3. Use absorbent paper to remove excess water from around the edge of the coverslip.
4. Dry the base of the slide before putting it on the stage of the microscope.
5. Observe the slide under the low and high powers of your microscope.

You may have to stain the preparation you have made by irrigating the slide. Place a drop of the stain (usually methylene blue or iodine) on one side of the coverslip and a piece of absorbent paper on the opposite side. As the paper absorbs water from underneath the coverslip, the stain spreads through. When the stain has spread right across the specimen, put a drop of water on the side of the coverslip and absorb the excess stain with absorbent paper. Wipe the base of the slide and check that the coverslip is dry.

Question 2 often involves making plan diagrams to show the distribution of tissues in a structure and making drawings of cells observed through the high-power objective of a microscope.

When you make a plan diagram, follow these rules:

- hold up the slide and look at it with the naked eye to see the specimen before placing it on the stage of the microscope
- make the drawing fill at least half the space provided; leave space around the drawing for labels and annotations
- use a sharp HB pencil (never use a pen)
- use dots or faint lines to plan how you will make your drawing
- use thin, single, unbroken drawing lines ('clear and continuous lines'), *not* 'feathery' lines
- show the outlines of the tissues
- make the proportions of tissues in the diagram the same as in the section
- do *not* include drawings of cells
- do *not* use any shading or colouring.

Add labels and annotations (notes) to your drawing *only* if you are asked for these in the question. Use a pencil and a ruler to draw straight lines from the drawing to your labels and notes. Write labels and notes in pencil so you can alter your answer if necessary.

You may be asked to make drawings of plant cells, such as epidermal cells, xylem vessel elements, companion cells or phloem sieve tube elements. If so, follow the general advice above and:

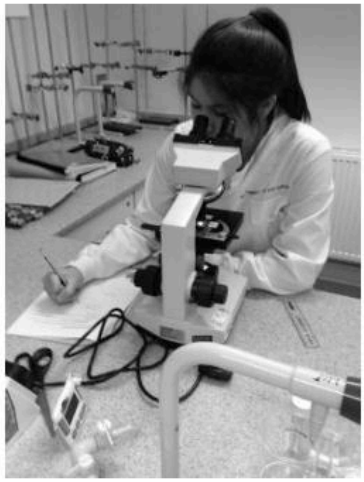
- search the slide to find a suitable group of cells to draw to answer the question
- if not told how many cells to draw, choose three or four
- plan on the paper where you will draw the cells and put short lines to indicate the width and length of one of the cells
- draw a line to represent the *inside* of one of these cells
- look carefully at the thickness of the cell wall between the cells
- make some faint dots on the paper to indicate where to draw the inside of the adjacent cells and then draw these with clear, continuous lines
- draw in the middle lamella, which is where the cell walls of adjacent cells are joined together

Link

Look at the Practical skills section 'Resolution and magnification' in Unit 1 and 'Observing the mitotic cell cycle' in Unit 5.

Link

There are some plan diagrams in the Practical skills sections of Unit 7 (pages 74–75) and Unit 8 (pages 89–90). These plan diagrams have been shaded to help show the different tissues. Your plan diagrams should not be shaded – see the answer to (a) on page 106 (Unit 9).



▲ **Figure 20.5** A student using a microscope correctly to make drawings. She is right-handed so has placed her paper on her right-hand side. If you are left-handed, then place your paper on the left of the microscope when drawing

Exam tip

Make sure that your label lines, labels and annotations are horizontal. Do not draw label lines that cross each other.

- look carefully at the question to see what you are required to do. If you are not asked to label your drawing, no marks will be gained for including labels.

Question 2 often asks for comparisons between two structures: usually the specimen you have drawn from a slide and a photograph. Comparisons are best shown in a table.

When drawing, make sure that you are not drawing something that you remember should be present and bear in mind the resolution of the light microscope.

Link

Question 2 in Exam Paper 3 on page 255 is an example of a comparison question.

Advice for Paper 3

Remember these tips for taking Paper 3:

- read through each question carefully before you start any practical work
- annotate the questions as you read them and highlight the topic areas from the syllabus so you know what knowledge to use when answering questions that ask you to interpret and conclude
- keep one area of the lab bench for your exam paper and keep it dry; do not put any liquids near the paper
- look at the time to make sure that you spend about one hour on each question (assuming that there are two questions on the paper)
- prepare your results table in pencil and plan what you are putting into the columns and rows carefully before you start
- write your results into the table; do not write them down somewhere else on the paper and write them in later
- hold up the microscope slide and look at it with your eyes before placing it on the stage of the microscope
- plan out what you have to draw in the spaces provided
- draw, label and annotate with a sharp pencil
- leave yourself enough time to look through your paper to double check your calculations and make sure that you have answered all the questions.

Exam tip

When you make a table of comparison, put similarities running across the columns and differences in separate columns. Make direct comparisons when giving differences.

Exam tip

When you take Paper 3, make sure that you have: a calculator, two sharpened HB pencils, a pencil sharpener, a clean eraser and a clean, complete plastic ruler.

Paper 5 (30 marks)

This paper is set on the whole syllabus. The questions involve aspects of planning, analysis and evaluation. There is no practical work, so the paper is not taken in a lab. The paper is set so that marks are awarded for the following skills:

Planning (P)

- Defining the problem (5 marks)
- Methods (10 marks)

Analysis, conclusions and evaluation

- Dealing with data (8 marks)
- Evaluation (4 marks)
- Conclusions (3 marks)

All the sections in this book that are about practical skills are relevant to Paper 5. The only skill not so far discussed in detail is the Planning skill.

Paper 5 tests your ability to devise hypotheses, make predictions, plan experiments, interpret results and evaluate procedures and data. Planning and implementing your own plans during your course will provide you with good practice at these skills. The contexts of questions in Paper 5 may involve unfamiliar experiments or biological materials. Do not panic, if you have revised thoroughly all the topics in the syllabus and practised writing and implementing your own plans, then you have the experience to answer confidently.

Planning

You will be expected to plan a logical procedure as part of an investigation that someone else could carry out following your instructions.

Here is some advice about answering the planning question on Paper 5.

Identify the independent, dependent and control variables for the investigation. Identify these variables in your plan. You choose the range of the **independent variable** (IV) and the intermediate values across the range. In some investigations there are two or more IVs; for example, in an investigation to determine the effect of temperature on the uptake of glucose by yeast cells at two different values of pH.

The **dependent variable** (DV) is the variable that you set out to observe and/or measure. You need to give a detailed explanation of how you will use apparatus to measure the DV and how you will keep at least one important **control variable** constant.

Choose apparatus and materials that are appropriate. Only choose apparatus that is available in a school or college lab, not sophisticated apparatus (e.g. an electron microscope) that is not.

Decide whether any **control experiments** are needed. For example, you may need a control experiment to show that an organism is responsible for the changes that you measure. A control experiment is set up in exactly the same way but uses inert material of the same volume or mass to replace the living organism.

Write the procedure (method) using logical **numbered steps** as a set of instructions; do *not* use continuous prose. This allows you to include instructions such as 'repeat step 5'. Describe how to collect the results. Comment on the need to repeat the experiment at least twice to ensure that results are repeatable and that anomalous results can be identified.

Carry out a risk assessment. Identify the main hazard(s) and state appropriate safety precautions for each one. If necessary, include a description of how to dispose of hazardous materials at the end of the investigation.

Analysis, conclusions and evaluation

All the information given for Paper 3 (see pages 240–241) is relevant to Paper 5. In addition, you should know how to use descriptive statistics and how and when to use four statistical tests. You should also know the criteria for using each of these four tests:

chi-squared test

t-test

Spearman's rank correlation coefficient test

Pearson's linear correlation coefficient test.

Link

Descriptive statistics and these four statistical tests are described on the support website.

Exam tip

At the end of Units 12–19 there are two questions that deal with data analysis, evaluation and planning. Try them and compare your answers to those given on the support website.

Exam tip

In the examination, the planning question will be based on a topic that you have studied from Units 1 to 19 and some practical work that you should have done. If the context of the investigation or apparatus required is unfamiliar then sufficient details will be provided.

Link

See Units 2, 3, 4, 12 and 13 for examples of procedures given as numbered steps. Practise writing procedures like this for different investigations.

Exam tip

Make your risk assessment specific. In the exam you only need to identify the most significant risk. Do not include good practice that applies to all lab work (e.g. wear a lab coat, tie back loose hair, etc.).

21 Exam-style questions

Paper 1 style questions

Paper 1 is set on the AS Units 1 to 11. There are no multiple choice questions (MCQs) in Papers 4 and 5.

The MCQs in Paper 1 test your recall of knowledge and your ability to apply your knowledge to familiar and unfamiliar contexts.

Each question has four options, **A**, **B**, **C** and **D**. Only one of these options is the correct answer. The other options are known as distractors. You write your answers on a special answer sheet using a pencil. There is one mark for each question and the paper has 40 questions.

Exam tip

In Type 1 MCQs the options **A**, **B**, **C** and **D** appear directly below the question, the options in each question are always presented in the same way and can be phrases or complete sentences, or presented in a table, graph or diagram.

1 There is considerable variation in the diameter of living cells. The diameter of a cell of *Saccharomyces cerevisiae*, a single-celled fungus, is 600 nm. The diameter of a human red blood cell is 7.2 µm.

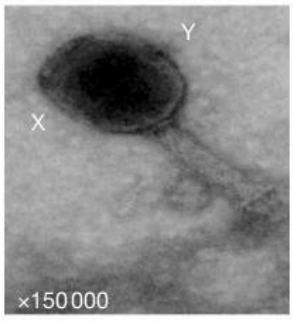
How much greater is the diameter of the red blood cell compared with the diameter of *S. cerevisiae*?

A 0.012 B 0.12
C 1.2 D 12

Exam tip

Question 1 cannot be answered just by knowledge alone. You have to work out the answer from the information given.

2 The photograph shows a virus particle.



What is the diameter of the virus between points **X** and **Y**?

A 25 nm B 25 µm
C 70 nm D 70 µm

3 A fine glass tube was inserted into a phloem sieve tube. Phloem sap continued to drip from the glass tube for several hours. Which is responsible for this?

A active transport B hydrostatic pressure
C mass flow D transpiration pull

Exam tip

In Type 2 MCQs the options (**A**, **B**, **C** and **D**) are preceded by terms or statements, usually marked as 1, 2, 3, etc. Each option is a particular combination of the numbered statements (e.g. 1 and 2 only, 3 and 4 only) but only one of the options has the correct combination, the others are distractors.

4 A student prepared some test-tubes with solutions as follows:

Test-tube	Contents
1	glucose and sucrase
2	glycogen and amylase
3	starch and amylase
4	starch and sucrase
5	sucrose and amylase

All the test-tubes were kept at 40 °C for 15 minutes. In which test-tubes would reducing sugar be detected after 15 minutes?

A 1, 2 and 3 only B 3 and 4 only
C 2, 3, 4 and 5 only D 1, 2 and 5 only

5 There are numerous metabolic pathways in animal cells. Each reaction in a particular pathway is catalysed by a specific enzyme. The rate of one of the reactions is controlled.

Which may be involved in the control of such a reaction?

1 a change in enzyme concentration

2 a change in substrate concentration

3 inhibition by the final product of the reaction

A 1 only B 3 only

C 1 and 2 only D 1, 2 and 3

6 Which is **not** a role of mitosis?

A cell repair B cell replacement

C growth D wound healing

★ Exam tip

Notice that Question 6 has the word **not** in it. Paper 1 may have a few questions of this type.

7 During transcription, nucleotides are assembled along the template strand of DNA to form mRNA.

In a DNA molecule, the triplet CAG on the template strand codes for the amino acid valine.

What is the base sequence of the codon on the mRNA that corresponds to the DNA triplet for valine?

A CAG B CUG

C GTC D GUC

8 Monoclonal antibodies are made by cells known as hybridomas.

Which event occurs during the production of a hybridoma?

A attachment of antigens to complementary B-lymphocyte receptors

B fusion of activated B-lymphocytes with myeloma cells

C protein synthesis to make a specific antibody molecule

D insertion of genes for a specific antibody into a myeloma cell

9 Which row shows the component molecules of the four macromolecules shown?

	Amylopectin	Nucleic acid	Phospholipid	Polypeptide
A	α-glucose	organic bases	two fatty acids and phosphate	amino acids
B	β-glucose	amino acids	cholesterol and three fatty acids and phosphate	nucleotides
C	α-glucose	nucleotides	glycerol, fatty acids and phosphate	amino acids
D	β-glucose	phosphate and pentose sugar	glycerol, phosphate and three saturated fatty acids	carboxylic acids

★ Exam tip

Use a ruler to help you analyse tables like the one above. It will help you concentrate on one column or one row of information at a time and not be distracted by what is elsewhere in the table.

10 Water is a solvent in living organisms. Which row shows substances that will dissolve in water?

	Carbon dioxide	Fatty acids	Glucose	Oxygen	Sodium ions
A	✓	✗	✓	✓	✓
B	✗	✓	✓	✗	✓
C	✗	✓	✗	✓	✗
D	✓	✗	✗	✗	✓

Paper 2 style questions

1 (a) The table contains statements about four molecules.

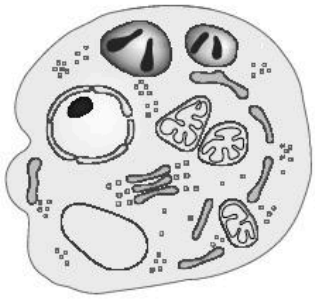
Complete the table by putting a tick (✓) or a cross (✗) in each box of the table.

Statement	Haemoglobin	DNA	Phospholipids	Antibodies
contains iron				
contains phosphate				
hydrogen bonds stabilise the molecule				
hydrolysed to amino acids				

[4]

[Total: 4]

2 The drawing here was made from transmission electron micrographs of the feeding form of *Plasmodium falciparum*, the causative agent of malaria.



(a) Use the information in the drawing to explain why *P. falciparum* is a eukaryotic organism. [2]

(b) (i) The most common method of transmission of *P. falciparum* is by female *Anopheles* mosquitoes.

Describe how *P. falciparum* is transmitted in this way. [2]

(ii) State **two** other ways in which *P. falciparum* can be transmitted. [2]

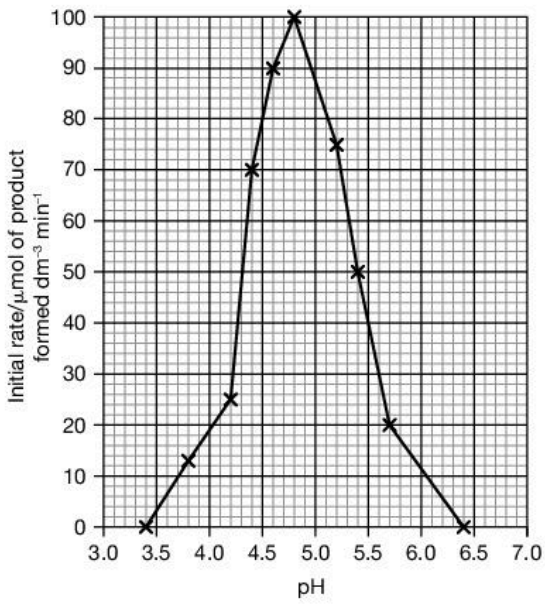
(iii) Describe **two** precautions that people can take to avoid being infected by *P. falciparum*. [2]

(c) It has proved very difficult to develop a vaccine to provide protection against malaria.

Explain the biological problems in developing such a vaccine. [4]

[Total: 12]

3 A student investigated the effect of pH on the activity of the enzyme β-glucosidase, which catalyses a reaction in the breakdown of cellulose. The student used buffer solutions to give a range of values of pH. The temperature was kept constant at 30 °C. The results are shown in the graph.



(a) Use the graph to describe the effect of pH on the activity of β-glucosidase. [3]

(b) Explain the effect of pH on the activity of β-glucosidase. [4]

(c) Draw a curve on the graph to show the results you would expect if the investigation was repeated in exactly the same way but at a temperature of 20 °C. [2]

(d) The student repeated the investigation with maltase, which catalyses the same type of reaction in the breakdown of amylose. No reaction occurred at any pH.

Explain why maltase did not catalyse the same reaction as β-glucosidase. [2]

[Total: 11]

4 (a) The table shows some information about the composition of blood, tissue fluid and lymph.

	Blood	Tissue fluid	Lymph
red blood cells	present		
white blood cells			very few
plasma proteins		very few	
haemoglobin		absent	

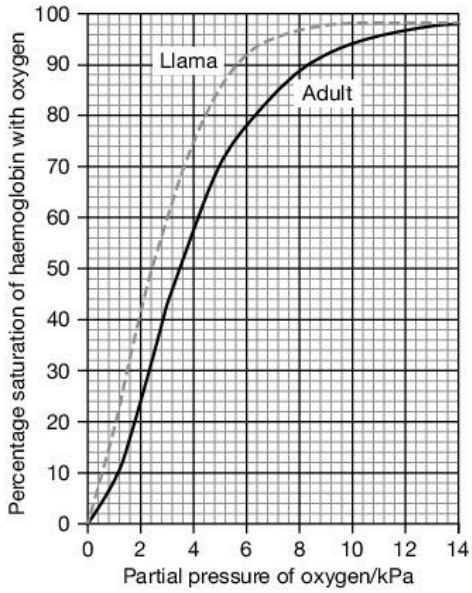
Complete the table. [4]

Exam tip

In (b)(ii) look at the right-hand side of the graph which represents 'loading' with oxygen in the lungs and the left-hand side which represents 'unloading' in the respiring tissues, e.g. in the muscles.

(b) The llama, *Lama glama*, is a mammal adapted to live at high altitudes in the Andes in South America. The animal is often found at altitudes higher than 5000 metres above sea level.

The graph shows the oxygen haemoglobin dissociation curves for a llama that lives at high altitude and an adult human at sea level.



At 5000 metres the partial pressure of oxygen in the lungs is about 6 kPa.

(i) Use the graph to state the percentage saturation of haemoglobin with oxygen at a partial pressure of oxygen of 6 kPa for both the human and the llama. [1]

(ii) Explain the advantage of the dissociation curve for the llama being to the left of that for the adult human. [3]

(iii) State and explain how humans adapt to survive when they travel from sea level to stay at high altitude. [4]

[Total: 12]

5 A layer of stem cells is found beneath the epidermis of the skin.

(a) Explain why it is important that there are stem cells in the skin. [2]

(b) Epidermal growth factor (EGF) is a small polypeptide that acts as a cell signalling compound. EGF binds to receptors on the surface of stem cells and stimulates the cells to divide.

(i) State why EGF cannot pass through the membrane to stimulate processes within the cell. [1]

(ii) Describe the likely effects of EGF on the cell cycle of stem cells. [3]

(c) Panitumumab is a monoclonal antibody that is used in the treatment of colon cancer. This monoclonal antibody is produced by hybridoma cells formed from B-lymphocytes from a mouse injected with receptor molecules for human EGF.

Exam tip

Notice that (c)(ii) asks about *treatment* not diagnosis.

(i) State how hybridoma cells are formed from B-lymphocytes. [1]

(ii) Explain how treatment with panitumumab is likely to be effective. [2]

[Total: 9]

Paper 3 style questions

You can try this example of a Paper 3 in a school or college laboratory or at home. All the materials and apparatus that you need are listed in the paper. Question 2 usually has one or more microscope slides and one or more photographs. This paper has only photographs and so is not representative of a real Paper 3.

Instructions for making the 1.0 mol dm^{-3} solution of sucrose for Question 1 are in Unit 20 Practical assessment page 234.

Before you start any practical work, read carefully through the **whole** of the paper. Plan the use of the **two hours** to make sure that you finish all the work that you would like to do.

If you have enough time, think about how you can improve the accuracy of your results, for example by obtaining and recording one or more additional measurements.

Exam tip

As you read through Question 1 when in the exam, imagine you are carrying out the practical and look for all the apparatus you will use that should be set out on the lab bench for you.

1 The water potential of a plant tissue is a function of the solute potential and the pressure potential of the cells. The water potential gradient between a plant tissue and any solution in which it is immersed determines the net movement of water between the tissue and the solution.

You are required to investigate the effect of immersing pieces of potato, *Solanum tuberosum*, in different sucrose solutions.

You are provided with the following:

- 100 cm^3 of 1.0 mol dm^{-3} sucrose solution in a suitable container
- water for diluting the sucrose solution in a suitable container
- two large potatoes
- a sharp knife and board for cutting
- a transparent, plastic ruler and one piece of 2 mm graph paper
- a measuring cylinder or measuring beaker or cup with graduations every 20 cm^3
- seven beakers, mugs, plastic cups or polystyrene cups as containers for immersion of potato strips
- a marker pen
- paper towels.

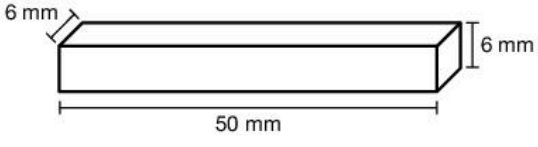
Proceed as follows:

1 Use the 1 mol dm^{-3} sucrose solution to prepare five different concentrations of sucrose by **simple (proportional) dilution**. You will need to prepare *at least* 200 cm^3 of each concentration.

(a) Prepare a table to show how you will make the solutions. [4]

2 Put *at least* 200 cm^3 of each sucrose solution into the containers.

3 Use the sharp knife to cut the potato into strips with the dimensions shown in the diagram. You will need *at least* 10 strips. Make sure that there is no peel left on the strips of potato and that the ends of each strip are cut square.



4 Place *at least* two strips of potato into each solution of sucrose. Make sure that the strips are completely immersed. Put the beakers or plastic cups to one side for 30 minutes. While you are waiting continue with the rest of the questions starting from **(f)** or start **Question 2**.

5 After 30 minutes remove the strips of potato from the solutions of sucrose and measure their lengths.

(b) Prepare a space to record your results.

Record all your results and any processed data in the space you have prepared. [4]

(c) State the uncertainty of the measurements that you have taken and explain your answer. [2]

(d) Explain how you could use your results to estimate the water potential of the potato tissue. [3]

(e) State **two** significant sources of error in your investigation and explain the effect that each has on the results. [2]

The epidermis was peeled from some onion scale leaves, cut into pieces and placed in solutions of different concentration of sodium chloride. The pieces were immersed for 10 minutes and then placed on microscope slides in the same bathing solutions and covered by cover slips.

The number of cells showing signs of plasmolysis in 100 cells chosen at random was counted. The results are shown in the table.

Concentration of sodium chloride solution / mol dm^{-3}	Water potential / kPa	Percentage of plasmolysed cells
0.00	0	0
0.10	−440	7
0.20	−850	43
0.30	−1260	67
0.40	−1680	87
0.50	−2120	100

(f) Plot a graph of these results. [4]

(g) The solute potential of plant cells is estimated as the value when 50% of the cells are plasmolysed.

(i) State the water potential at which 50% of the cells are plasmolysed. [1]

(ii) Explain why it is possible to estimate the solute potential of plant cells using this method. [3]

[Total: 23]

Exam tip

As you carry out the practical, identify any likely errors in measurement and any limitations of the procedure (see Unit 20, pages 240–241). Write down any ideas on any white space in your exam paper so you can use them in your answers.

Exam tip

Make sure you follow the rules for plotting graphs (see page 239); use a sharp pencil to make small saltire crosses and a ruler to draw lines between the points.

2 (a) Figure 1 is a photomicrograph of a cross section through part of a leaf of black hellebore, *Helleborus niger*.

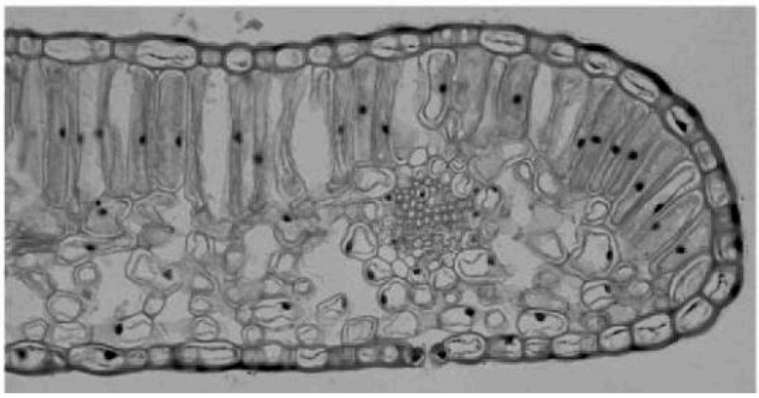


Figure 1 × 200

Draw a large, labelled plan diagram of the section of the leaf shown in Figure 1.

You should draw the correct shape and proportions of the different tissues. [6]

(b) (i) The cell walls surrounding many of the palisade mesophyll cells in Figure 1 are clearly visible.

Calculate the mean **length** of the palisade mesophyll cells shown in Figure 1 in micrometres (μm).

Indicate clearly on Figure 1 the palisade mesophyll cells which you have used in your calculation.

Show all your working. [4]

(ii) State **one** way in which you standardised your measuring of the palisade mesophyll cells. [1]

★ Exam tip

Show your working clearly using words and figures. A table is a good way to show the working for (b)(ii).

(c) Figure 2 is a photomicrograph of a stained cross section through part of a leaf of a different species.

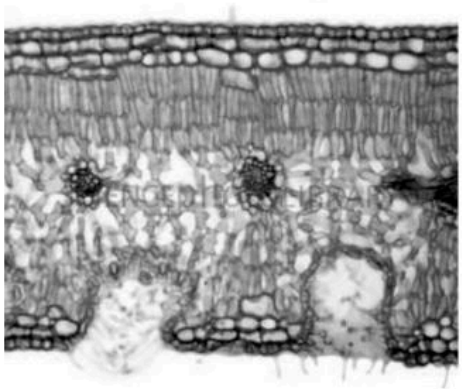


Figure 2 × 100

Study carefully the structure of the leaf shown in Figure 2.

Prepare a space that is suitable for you to record the observable differences between the **structure** of the leaves shown in Figures 1 and 2.

Record your observations in the space you have prepared. [6]

[Total: 17]

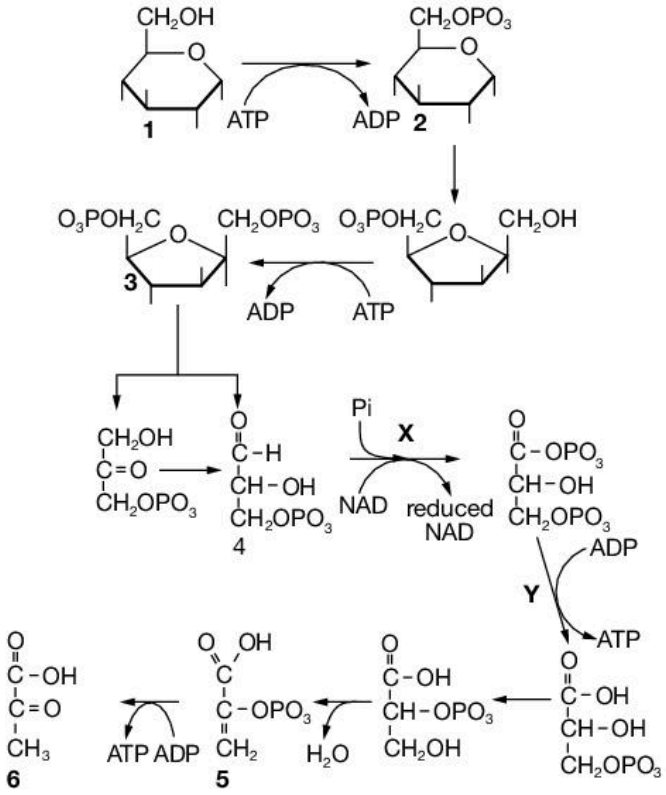
★ Exam tip

This means draw a table. You may find it helpful to have three columns headed 'feature', 'leaf in Figure 1' and 'leaf in Figure 2'.

Paper 4 style questions

Section A

1 The diagram shows the stages of glycolysis in a mammalian cell.



Exam tip

Look carefully at the diagram stage by stage before you read the questions.

(a) (i) Name the molecules labelled 2, 3, 4 and 6. [4]

(ii) State a possible source of substance 1. [1]

(iii) State what happens at stage Y. [1]

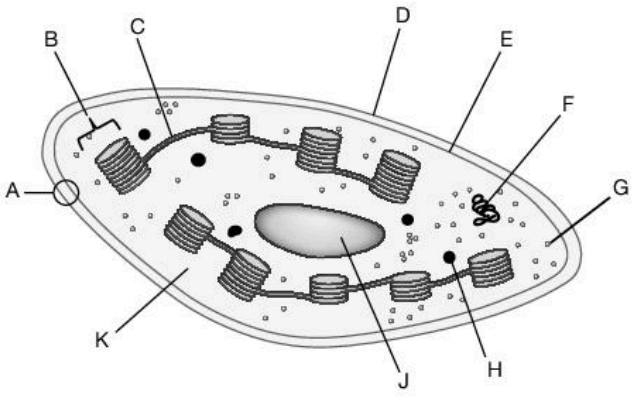
(iv) State what happens to the reduced NAD. [2]

(b) (i) Discuss the importance of the reaction that occurs at X. [2]

(ii) State the immediate fate of the molecule labelled 6 under aerobic conditions in the mammalian cell. [2]

[Total 12]

2 (a) The drawing was made from electron micrographs of chloroplasts.



Magnification = ×2300

The table shows six processes that occur in chloroplasts.

Complete the table by naming the part of the chloroplast that carries out each process and identifying it from the diagram using one of the letters, **A** to **K**.

Function	Name	Label from diagram
absorption of light by chlorophyll		
fixation of carbon dioxide		
production of oxygen		
translation of chloroplast genes		
storage of polysaccharide molecules		
transcription of chloroplast genes		

(b) Phosphate ions move into chloroplasts through carrier proteins in the chloroplast envelope.

Explain why chloroplasts require a constant supply of phosphate ions. [3]

[Total: 9]

3 The table shows the concentrations of glucose and insulin in the blood plasma at different times in the life of a teenager who often went without meals.

	Glucose concentration in the plasma / mg 100 cm^{-3}	Insulin concentration in the plasma / arbitrary units
during an overnight fast	80	9
during a large breakfast	160	70
after the absorption of a meal is complete	70	10
during prolonged fasting	60	6

★ Exam tip

Study the table carefully. Read each column and then read each row. You can make any observations or calculations in the white space on the exam paper.

(a) Describe the relationship between the concentrations of glucose and insulin in the blood plasma as given in the table. [2]

(b) Explain the changes in the concentrations of glucose and insulin in the blood during a meal and when it is absorbed. [5]

(c) Explain how the concentration of glucose is maintained during periods of prolonged fasting. [3]

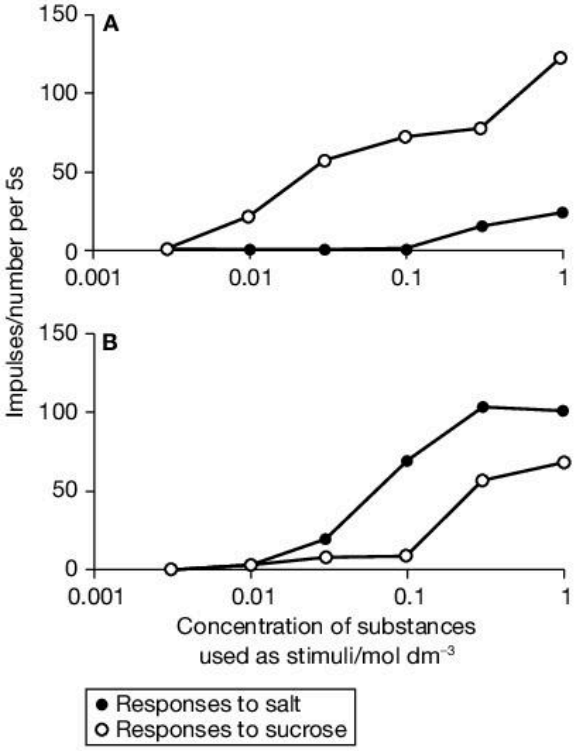
[Total: 10]

4 Chemoreceptors in the tongue respond to five different tastes: sweet, sour, bitter, salt and umami (savoury). The diagram shows a chemoreceptor exposed to a salt solution and the response at **X** of the sensory neurone that transmits impulses to the brain.

(a) State the advantage of having microvilli over the surface of the chemoreceptor. [1]

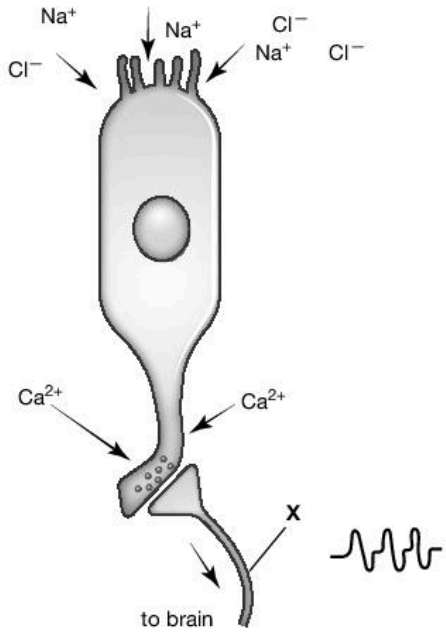
(b) Describe the function of calcium ions in the chemoreceptor. [3]

(c) The supply of sensory neurones to chemoreceptors is complex. Some sensory neurones have dendrites on several different chemoreceptors. The graphs show the responses of two different sensory neurones (**A** and **B**) to stimulation by increasing concentrations of salt and sucrose.



Use the information in the graphs to comment on the differences between the responses shown by neurones **A** and **B**. [4]

[Total: 8]



Exam tip

Study each graph carefully. Use a ruler to move across each graph from left to right. Make notes about the differences between them on any white space on the exam paper.

Section B

Answer one question

5 (a) Explain how meiosis and fertilisation give rise to genetic variation in populations of animals. [9]

(b) Discuss the roles of 'frozen zoos'. [6]

[Total: 15]

6 (a) Describe the Calvin cycle and discuss its role in the production of organic molecules in plants. [9]

(b) Discuss the role of botanic gardens in the restoration of degraded habitats. [6]

[Total: 15]

Exam tip

Make a brief plan for your chosen question. You could indicate which topics you are going to use in each paragraph.

Paper 5 style questions – Planning, analysis and evaluation

1 A student investigated the effect of different wavelengths of light on the rate of the light dependent stage of photosynthesis.

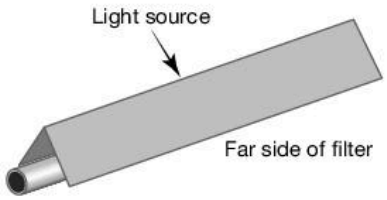
Dichlorophenolindophenol (DCPIP) is a redox dye that is used to determine the activity of the light dependent stage of photosynthesis. The blue colour of DCPIP disappears as it is reduced:

electrons and hydrogen ions + DCPIP (blue) → reduced DCPIP (colourless)

The student prepared a suspension of chloroplasts in a cold, sucrose solution buffered at pH 7.

Samples of the chloroplast suspension were mixed with a DCPIP solution and kept in the dark.

A short length of capillary tubing was dipped into the mixture of chloroplast suspension and DCPIP so that the mixture was taken up. The capillary tubing was placed on a bench beneath a coloured filter as shown in the diagram.



The student timed how long it took for the DCPIP to decolourise. The student then repeated the procedure using filters of different colours.

(a) Identify the independent and dependent variables in this investigation. [2]

(b) Explain why the chloroplast suspension was prepared in a sucrose solution. [2]

(c) Suggest **two** suitable controls to include and explain why you think each is necessary. [4]

(d) Explain how the student would calculate the rate of the light dependent stage of photosynthesis from the results collected. [1]

(e) The student decided to use the same procedure to investigate the effect of light intensity on the rate of the light dependent stage of photosynthesis.

(i) State a hypothesis that the student could test. [1]

(ii) Write a plan that the student could follow to test the hypothesis and gain valid, high-quality data.

Your method should be set out in a logical way and be detailed enough to allow another person follow it. [7]

[Total: 17]

★ Exam tip

The investigations described in Paper 5 may be familiar or unfamiliar. Read through carefully and try to imagine carrying them out in the lab or in the field. This should help you answer questions like Question 1 (c).

2 The marine iguana, *Amblyrhynchus cristatus*, is endemic to the Galápagos Islands. On the island of Santa Cruz, many female iguanas congregate at one site on the coast to lay their eggs. This site provides the right conditions for egg laying, but is not colonised by adults as it provides them with little protection. The young iguanas begin to hatch from the eggs during May and June each year and feed on the algae that are exposed at low tide.

Researchers used the mark-release-recapture method to estimate the numbers of young iguanas on four occasions during May and June 1995. Each marking period lasted for two days, followed by one day of recapture.

(a) Suggest **two** factors that the researchers should consider when using mark-release-recapture to estimate the population of the young marine iguanas. [2]

The table shows the results.

Date	Number marked and released	Number recaptured	Number marked recaptured	Population estimate
10 May	237	232	101	544
25 May	539	354	214	892
10 June	195	169	100	
25 June	128	124	103	154

(b) Complete the table by calculating the population estimate for the 10th June.
Write out the formula that you will use for calculating the population and give your answer to the nearest whole number. Show your working. [3]

(c) Suggest possible reasons why the estimated population on the 25th June was much less compared with the other estimates. [2]

Adult marine iguanas swim out to sea to feed on algae growing on rocks down to a depth of 15 m. Researchers investigated the relationship between food availability and the maximum size of adult iguanas on 16 of the islands in the Galápagos. They measured the height of the algae around each island and the body length of a sample of the largest adults.

The researchers tested the hypothesis that there was a correlation between the mean height of algae and the maximum length of adult iguanas by calculating the Pearson's linear correlation coefficient (r).

(d) Pearson's correlation coefficient can only be used if the data satisfies certain criteria. State **two** of these criteria. [2]

(e) The researchers calculated the value of r as 0.76.
The table shows some of the critical values for Pearson's linear correlation coefficient.

Number of pairs of measurements	Critical values	
	p = 0.05 (5%)	p = 0.01 (1%)
14	0.532	0.661
15	0.514	0.641
16	0.497	0.623

(i) State what this value indicates about the relationship between the mean height of algae and the maximum length of adult iguanas. [1]

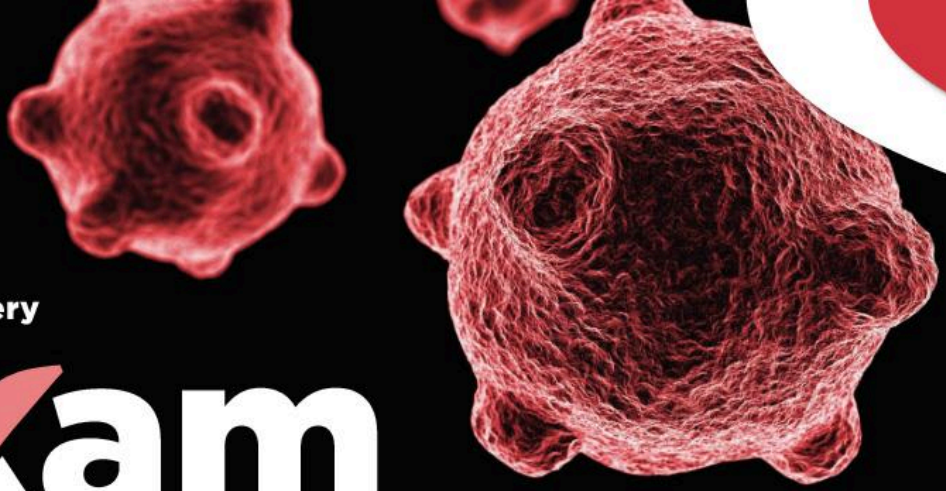
(ii) Describe how the researchers used this table to find out if the value for r = 0.76 is significant. [3]

[Total: 13]

★ Exam tip

This question shows why it is important to know *when* you can use the different statistical tests and *how* you interpret the results.

Index



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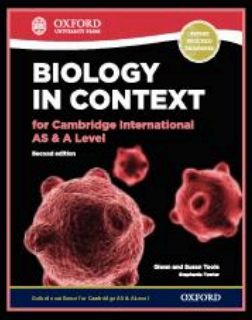

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