

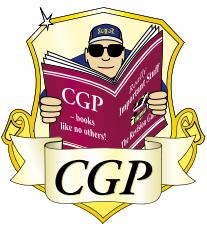
AQA

A-Level **Biology**

Exam Board: AQA

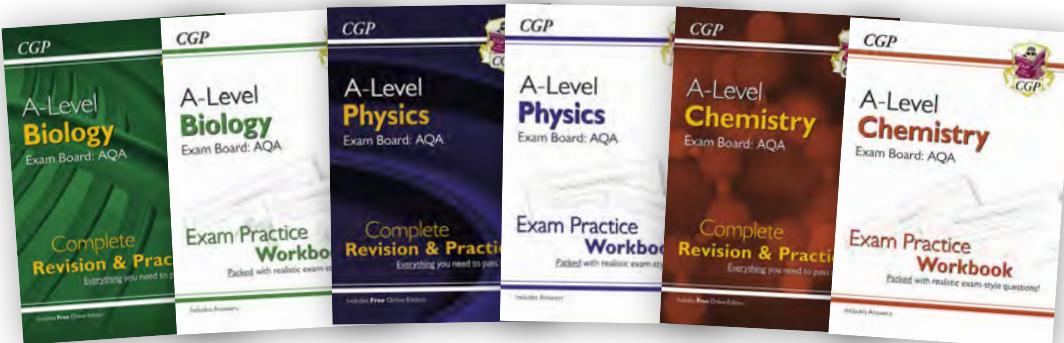
Student Book

The Complete A-Level Course for AQA



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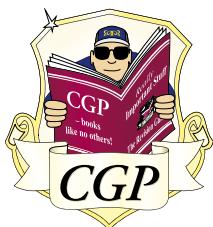
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A-Level Biology

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Let's face it, Biology is a tough subject. You'll need to get to grips with a lot of difficult concepts, and have plenty of practical skills up your lab-coat sleeve.

But don't worry — this brilliant CGP book covers everything you'll need for both years of the AQA course. It's packed with clear explanations, exam practice, advice on maths skills and practical investigations... and much more!



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How to use this book

Learning Objectives

- These tell you exactly what you need to learn, or be able to do, for the exams.
- There's a specification reference at the bottom that links to the AQA specification.

Maths Skills Examples

There's a range of maths skills that you could be expected to apply in your exams. Examples that show these maths skills in action are marked up like this. There's also a Practical and Maths Skills section at the front of the book.

Exam Tips

There are tips throughout the book to help with all sorts of things to do with answering exam questions.

Cancer

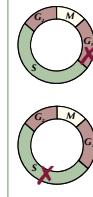
Mitosis and the cell cycle are controlled by genes. Normally, when cells have divided enough times to make enough new cells, they stop. But if there's a mutation in a gene that controls cell division, the cells can grow out of control. The cells keep on dividing to make more and more cells, which form a tumour. Cancer is a tumour that invades surrounding tissue.

Cancer treatments

Some treatments for cancer are designed to control the rate of cell division in tumour cells by disrupting the cell cycle. This kills the tumour cells. These treatments don't distinguish tumour cells from normal cells though — they also kill normal body cells that are dividing. However, tumour cells divide much more frequently than normal cells, so the treatments are more likely to kill tumour cells.

Examples

Some cell cycle targets of cancer treatments include:



G₁ (cell growth and protein production)

Some chemical drugs (chemotherapy) prevent the synthesis of enzymes needed for DNA replication. If these aren't produced, the cell is unable to enter the synthesis phase (S), disrupting the cell cycle and forcing the cell to kill itself.

S phase (DNA replication)

Radiation and some drugs damage DNA. At several points in the cell cycle, the DNA in the cell is checked for damage. If severe DNA damage is detected, the cell will kill itself — preventing further tumour growth.

Practice Question — Application

Q1 Methotrexate and vincristine are drugs used to treat cancer. Methotrexate blocks the formation of nucleotides within cells and vincristine prevents the formation of spindle fibres within the nuclei of cells. Which stage of the cell cycle is disrupted by:
a) methotrexate b) vincristine?

Practice Questions — Fact Recall

- Q1 What is the cell cycle?
- Q2 Why is mitosis needed?
- Q3 In what stage of the cell cycle does all the DNA unravel?
- Q4 Describe what happens during prophase.
- Q5 Describe what happens during telophase.
- Q6 What is cytokinesis?
- Q7 What is cancer?

Tip: Mutations are changes in the base sequence of an organism's DNA (see page 223).

Tip: Cancer is basically uncontrolled cell division.



Figure 7: Cancer of the knee — the tumour is sticking out of the leg.

Tip: Rapidly dividing cells, like hair cells and cells in the gut, are often affected by cancer treatments. This can cause side effects like hair loss.

3. Analysis of Cell Components

Investigating cells, and what's in them, involves digging out your microscope.

Magnification and resolution of microscopes

We all know that microscopes produce a magnified image of a sample, but resolution is just as important...

Magnification

Magnification is how much bigger the image is than the specimen (the sample you're looking at). It's calculated using this formula:

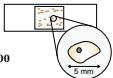
$$\text{magnification} = \frac{\text{size of image}}{\text{size of real object}}$$

Examples — Maths Skills

Calculating magnification

If you have a magnified image that's 5 mm wide and your specimen is 0.05 mm wide the magnification is:

$$\text{magnification} = \frac{\text{size of image}}{\text{size of real object}} = \frac{5}{0.05} = \times 100$$



Calculating size of image

If your specimen is 0.1 mm wide and the magnification of the microscope is $\times 20$, then the size of the image is:

$$\text{size of image} = \text{magnification} \times \text{size of real object} = 20 \times 0.1 = 2 \text{ mm}$$

Calculating size of real object

If you have a magnified image that's 5 mm wide and the magnification is $\times 50$, then the size of the real object (i.e. the size of the specimen you're looking at) is:

$$\text{size of real object} = \frac{\text{size of image}}{\text{magnification}} = \frac{5}{50} = 0.1 \text{ mm}$$

When you're calculating magnification you need to make sure that all lengths are in the same unit, e.g. all in millimetres. When dealing with microscopes these units can get pretty tiny. The table below shows common units:

| To convert | Unit | How many millimetres it is: | To convert |
|---------------|------------------------------|-----------------------------|-------------|
| $\times 1000$ | Millimetre (mm) | 1 mm | $\div 1000$ |
| $\times 1000$ | Micrometre (μm) | 0.001 mm | $\div 1000$ |
| $\times 1000$ | Nanometre (nm) | 0.000001 mm | $\div 1000$ |

The table shows that millimetres are three orders of magnitude (10^3 or 1000) times bigger than micrometres, which are three orders of magnitude bigger than nanometres.

Example — Maths Skills

To convert from a smaller unit to a bigger unit you divide by 1000.

So to convert 6 micrometres to millimetres you divide 6 by 1000

$= 0.006 \text{ mm}$. To go from a bigger unit to a smaller unit you times by 1000.

Examples

These are here to help you understand the theory.

Practice Questions — Application

- Annoyingly, the examiners expect you to be able to apply your knowledge to new situations — these questions are here to give you plenty of practice at doing this.
- All the answers are in the back of the book (including any calculation workings).

Practice Questions — Fact Recall

- There are a lot of facts you need to learn — these questions are here to test whether you know them.
- All the answers are in the back of the book.

6. Investigating Selection

You can carry out practical investigations into the effects of antimicrobial substances (substances that kill microorganisms, e.g. antibiotics, antiseptics or disinfectants) on microbes. These investigations should show you whether the microbes have evolved resistance to these substances or not.

Testing the effects of antibiotics

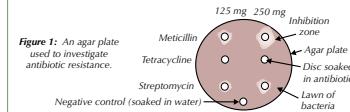
Antibiotics are medicines that are designed to kill bacteria. This makes them one of antimicrobial substances. You can investigate the effects of different antibiotics on bacterial growth using the following method. The whole investigation must be carried out using aseptic techniques.

These are explained on the next page. Read them through before you begin.

1. The bacteria you will use are likely to have been grown in a liquid broth (a mixture of distilled water, bacterial culture and nutrients).
2. Use a sterile pipette to transfer the bacteria from the broth to an agar plate (a Petri dish containing agar jelly). Spread the bacteria over the plate using a sterile plastic spreader.
3. Use sterile forceps to place paper discs soaked with different antibiotics spaced apart on the plate. Various concentrations of antibiotics should be used. You also need to add a negative control disc soaked only in sterile water.
4. Tape a lid onto the Petri dish (without completely sealing it), invert, and incubate the plate at about 25°C for 48 hours. This allows the bacteria to grow, forming a 'lawn'. Anywhere the bacteria can't grow can be seen as a clear patch in the lawn of bacteria. This is called an inhibition zone.
5. The size of an inhibition zone tells you how well an antibiotic works. The larger the zone, the more the bacteria were inhibited from growing.

Example

Figure 1 shows an agar plate after it has been incubated with paper discs soaked in the antibiotics meticillin, tetracycline and streptomycin.



- The tetracycline discs have no inhibition zones, so the bacteria are resistant to tetracycline up to 250 mg.
- The streptomycin discs have small inhibition zones, with the zone at 250 mg slightly larger than the one at 125 mg. So streptomycin inhibits the growth of some of the bacteria.
- The meticillin discs have the largest inhibition zones, so meticillin inhibits the growth of most of the bacteria.
- The negative control has no inhibition zone, which shows that the other results must be due to the presence of the antibiotics, not the paper disc.

Topic 4 — B: Diversity and Selection

Required Practicals

There are some key practicals that you'll be expected to do throughout your course. You'll need to know all about them for the exams. Information about these practicals is marked up with a Required Practical stamp.

Learning Objective:
Be able to use aseptic techniques to investigate the effect of antimicrobial substances on microbial growth.
(Required Practical 6).
Specification Reference 3.4.4

Tip: Make sure you carry out a full risk assessment before you carry out this practical. It's also really important that you understand how to use aseptic techniques properly before you start.

Tip: A negative control is not expected to have any effect on the experiment — see page 2 for more.

Tip: Don't completely seal the Petri dish before incubation — it will prevent oxygen from entering the dish, which may encourage the growth of anaerobic disease-causing bacteria. Don't open the dish after incubation.



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Practical Skills

There are some key practical skills that you'll not only need to use in your Required Practicals, but that you could be tested on in the exams too. These skills are covered in the Practical and Maths Skills section at the front of the book.

Tips

These are here to help you understand the theory.

Exam-style Questions

- Practising exam-style questions is really important — you'll find some at the end of each section.
- They're the same style as the ones you'll get in the real exams — some will test your knowledge and understanding and some will test that you can apply your knowledge.
- All the answers are in the back of the book, along with a mark scheme to show you how you get the marks.

Exam Help

There's a section at the back of the book stuffed full of things to help with your exams.

Glossary

There's a glossary at the back of the book full of all the definitions you need to know for the exams, plus loads of other useful words.

Exam-style Questions

- 1 The gills are the gas exchange organ in fish. Figure 1 shows a cross section through a dogfish gill.
- Figure 1
-
- A scanning electron micrograph showing a cross-section of a dogfish gill. The image reveals a dense, layered structure of gill tissue. An arrow labeled 'A' points to a specific feature within the tissue layers.
- 1.1 Name the structures labelled A in Figure 1 and explain how they increase the efficiency of gas exchange across the gills. (3 marks)
- 1.2 Give one other adaptation of the gills for efficient gas exchange. (1 mark)
- 1.3 Insects have a tracheal system for exchanging gases with the environment. Describe how oxygen gets into an insect's respiring cells. (2 marks)
- 1.4 Terrestrial insects lose water as a result of gas exchange. Explain two features insects have to reduce unwanted water loss. (2 marks)
- 2 Figure 2 shows a scanning electron micrograph of alveoli in a healthy human lung (left) and the effects of emphysema on the alveoli (right). The magnification is $\times 60$.
- Figure 2
-
- Two scanning electron micrographs labeled 'Figure 2'. The left image shows a healthy human lung with numerous small, rounded alveoli. The right image shows the effects of emphysema, where the alveoli are enlarged and fewer in number. An arrow labeled 'A' points to a specific alveolus in the healthy lung image.
- 2.1 Calculate the actual width of the labelled alveolus, A. Give your answer in μm . (2 marks)
- 2.2 Describe one difference between the healthy alveoli and the diseased alveoli, and explain what effect this would have on gaseous exchange in the alveoli. (3 marks)
- 2.3 Oxygen tents contain a higher percentage of oxygen than normal air. Suggest how being in an oxygen tent might benefit a patient with emphysema. (2 marks)

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Practical and Maths Skills

1. Planning an Experiment

You have to do practical work in class as part of your course. You'll be asked about it in exams too, so you need to know how to plan the perfect experiment.

Testing a theory

Before you start planning an experiment, you need to be clear about what you're trying to find out. Like all scientists, you should start off by making a **prediction** or **hypothesis** — a specific testable statement, based on theory, about what will happen in the experiment. You then need to plan a good experiment that will provide evidence to support the prediction — or help disprove it.

Example

Theory — photosynthesis requires light. The rate of photosynthesis is affected by light intensity.

Prediction — rate of photosynthesis will increase as light intensity increases.

Experiment — measure the rate of photosynthesis at various light intensities.

Exam Tip

At least 15% of your A-level Biology marks will come from assessment of Practical Skills in the exams — so you really do need to know this stuff.

Tip: The results of your experiment can't be used to prove that your theory is right, but can be used as evidence for or against it. There's more about what results mean on pages 15-18.

Getting good results

A good experiment is one that will give results that are:

- **Precise** — Precise results don't vary much from the mean. Precision is reduced by **random error** (the unpredictable way in which all measurements vary).
- **Repeatable and reproducible** — Repeatable means that if the same person repeats the experiment using the same methods and equipment, they will get the same results. Reproducible means that if someone different does the experiment, using a slightly different method or piece of equipment, the results will still be the same.
- **Valid** — Valid results answer the original question. To get valid results you need to control all the variables (see below) to make sure you're only testing the thing you want to.
- **Accurate** — Accurate results are really close to the true answer. **Human interpretation** of a measurement (e.g. determining a colour change) can **reduce** the accuracy of results.

Tip: Precise results are sometimes referred to as reliable results.

Here are some things you need to consider when designing a good experiment:

1. Variables

Variables are quantities that have the potential to change, e.g. temperature, pH. In an experiment you usually change one variable and measure its effect on another variable.

- The variable that you change is called the **independent variable**.
- The variable that you measure is called the **dependent variable**.

All the other variables should be controlled — when you're investigating a variable you need to keep everything else that could affect it constant.

This means you can be sure that only your independent variable is affecting the thing you're measuring (the dependent variable).

Exam Tip

Examiners love getting you to comment on experimental design or suggest improvements to methods — e.g. how a method could be improved to make the results more precise.

Example

For an investigation into how light intensity affects rate of photosynthesis:

- Light intensity is the independent variable.
- Rate of photosynthesis is the dependent variable.
- pH, temperature and the time the experiment is left for should all stay the same (and the quantities should be recorded to allow someone else to reproduce the experiment).

Exam Tip

If you get an exam question asking why a control is important in a particular experiment, make sure your answer is specific to that experiment (not just about why controls are good in general).

2. Controls

Negative controls are used to check that only the independent variable is affecting the dependent variable. Negative controls aren't expected to have any effect on the experiment.

Example

When investigating how light intensity affects the rate of photosynthesis, you should set up a negative control in which the experiment is carried out in the dark. No photosynthesis should happen with this control.

Positive controls can also be used. They should show what a positive result of the experiment should look like, to check that it is possible.

Example

If you're testing for the presence of glucose in a solution, you could carry out a Benedict's test. Before you start the experiment, you could test a solution that you know contains glucose to show what a positive glucose test result looks like. This is your positive control.

Tip: In a study with human participants, you should try to keep the variables of all the participants the same, e.g. they should all be the same age, sex, etc.

Tip: When testing a new drug to see if it works, the control group is given a placebo instead of the drug. A placebo is a dummy pill or injection that looks exactly like the real drug, but doesn't contain the drug. It's used to make sure that people don't improve just because they think they're being treated.

In studies, **control groups** are used. The subjects in the study are split into two groups — the experimental group and the control group. The control group is treated in exactly the same way as the experimental group, except for the factor you're investigating.

Example

If you were investigating the effect of eating a low sodium (salt) diet on blood pressure, you'd have two groups. One group would be the experimental group and be given a diet low in sodium. The other group would be a control group, who would be given a diet in which sodium wasn't reduced. This is done so that you can tell that any decrease in blood pressure is due to the low sodium diet and nothing else.

3. Repeats

Taking several repeat measurements and calculating the mean can reduce the effect of random error on your experiment, making your results more precise. Doing repeats and getting similar results each time also shows that your data is repeatable. This makes it more likely that the same results could be reproduced by another scientist in an independent experiment.

Example

For an investigation into how light intensity affects rate of photosynthesis, the experiment should be repeated at least three times for each light intensity used. A mean result should be calculated for each light intensity (see page 5).

Repeating measurements also reduces the likelihood that the results are due to chance — see next page.

4. Sample Size

Sample size is the number of samples in the investigation, e.g. the number of people in a drug trial. As with carrying out repeats, having a large sample size reduces the likelihood that the results are due to chance (e.g. if you get the same result twice it might be because of chance, but if you get it 100 times it's much more likely that it's not due to chance).

Tip: Scientists can use statistical tests to figure out if a result is likely to be due to chance or not. See pages 8-9 for more.

Taking accurate measurements

When you're planning an experiment you need to decide what it is you're going to measure and how often you're going to take measurements.

Example

If you're investigating the rate of photosynthesis, you could measure the volume of oxygen produced over time or the volume of carbon dioxide used over time. E.g. you could take measurements at 30 or 60 second intervals.

Then you need to choose the most appropriate apparatus, equipment and techniques for the experiment.

The measuring apparatus you use has to be sensitive enough to measure the changes you're looking for. For example, if you need to measure small changes in pH, a pH meter (which can measure pH to several decimal places) would be more sensitive than indicator paper.

The technique you use has to be the most appropriate one for your experiment. E.g. if you want to investigate plant cells undergoing mitosis, it's best to prepare a stained squash slide so you see the chromosomes clearly under the microscope (see page 90).



Figure 1: pH meters can be used to measure small changes in pH.

Risk assessments

In order to work safely, you need to carry out a risk assessment for your experiment. To do this, you need to identify:

All the dangers in the experiment.

For example, any hazardous chemicals, microorganisms or naked flames.

Who is at risk from these dangers.

This could be you and your lab partner, but it could also be anyone who is in the same room or building.

What can be done to reduce the risk.

You should wear a lab coat and safety goggles as a standard precaution, but you may need to take other safety precautions, such as:

- Wearing gloves, if your experiment involves substances that are likely to irritate the skin.
- Carrying out your experiment in a fume cupboard if it involves volatile chemicals (see page 277), and keeping flammable chemicals away from naked flames (e.g. Bunsen burner flames).
- Using aseptic techniques (page 234) if you are culturing microorganisms.



Figure 2: A scientist wearing eye protection, gloves and a lab coat to protect her while she works.

Ethical issues

You also need to consider any ethical issues in your experiment.

Example

If you're using living animals (e.g. insects) you must treat them with respect. This means handling them carefully and keeping them away from harmful chemicals, extreme heat sources and other things that might cause them physical discomfort.

2. Carrying Out an Experiment

As part of your A-level in Biology, you're expected to carry out Required Practicals and be familiar with the techniques and apparatus involved in each one (see page 19). You could be asked about the skills you've learnt in your exams.

Using the correct apparatus and techniques

Examiners could ask you about a whole range of different apparatus and techniques. Make sure you know how to use all the instruments and equipment you've come across in class and can carry out all the techniques too. Here are some examples of equipment you should be able to use:

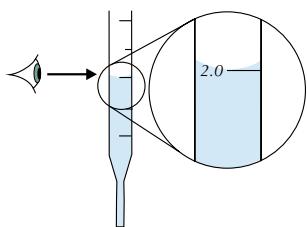


Figure 1: Measuring volume using the bottom of the meniscus.

Tip: A data logger (or data recorder) is an electronic device that can record data over time using a sensor. They can sometimes be connected to a computer.

Tip: You might need to record your results in a frequency table. These usually have three columns — the first gives the values or names of the different pieces of data, the second shows a mark (tally) for each piece of data, and the third shows the frequency, which you get by adding up the tally marks. E.g.

| species | tally | frequency |
|-----------|-------|-----------|
| sparrow | | 7 |
| blue tit | | 3 |
| blackbird | | 10 |

Examples

Measuring cylinders and graduated pipettes

These have a scale so you can measure specific volumes. Whichever one you use, make sure you read the volume from the bottom of the meniscus (the curved upper surface of the liquid) when it's at eye level — see Figure 1.

Water baths

Make sure you allow time for water baths to heat up before starting your experiment. Don't forget that your solutions will need time to get to the same temperature as the water before you start the experiment too. Also, remember to check the temperature of the water bath with a thermometer during the investigation to make sure it doesn't change.

Data loggers

Decide what you are measuring and what type of data logger you will need, e.g. temperature, pH. Connect an external sensor to the data logger if you need to. Decide how often you want the data logger to take readings depending on the length of the process that you are measuring.

You should also make sure you know how to do all the Required Practicals described in this book. You should be able to apply the techniques described in them to different contexts. E.g. page 107 describes how to prepare serial dilutions in order to find out the water potential of potato cells. You could also use serial dilutions to prepare solutions of varying substrate concentration in order to investigate the effect of substrate concentration on enzyme activity.

Recording data

As you get your results, you need to record them. It's a good idea to draw a **table** to record the results of your experiment in. When you draw a table, make sure you include enough rows and columns to record all of the data you need to. You might also need to include a column for processing your data (e.g. working out the mean — see next page). Make sure each column has a heading so you know what's going to be recorded where. The units should be in the column heading only, not the table itself — see Figure 2. The independent variable should be recorded in the left-hand column and the dependent variable in the right.

| heading | column | units |
|---|--|-------|
| Concentration of substrate / mol dm ⁻³ | Rate of reaction / cm ³ min ⁻¹ | |
| 0.2 | 10 | |
| 0.4 | 13 | |
| 0.6 | 17 | |

Figure 2: Table showing the rate of a reaction at three different concentrations of substrate.

If you're recording your data as decimals, make sure you do it to a consistent number of decimal places. When you're analysing your results, it makes sense to compare values that have been rounded to the same level of accuracy.

Anomalous results

When you look at all the data in your table, you may notice that you have a result that doesn't seem to fit in with the rest at all. These results are called **anomalous results**. You should investigate anomalous results — if you can work out what happened (e.g. you measured something totally wrong) you can ignore them when processing your results. However, you can't just exclude a value just because you don't like the look of it.

Tip: Doing repeats makes it easier to spot anomalous results.

3. Processing Data

Processing data means taking raw data and doing some calculations with it, to make it more useful. This is where your maths skills really come in.

Summarising your data

Once you've collected all your data, it's useful to summarise it using a few handy-to-use figures — like the mean and the range.

Exam Tip

At least 10% of your A-level Biology marks will come from assessment of maths skills in the exams.

Mean and range

When you've done repeats of an experiment you should always calculate a **mean** (a type of average). To do this add together all the data values and divide by the total number of values in the sample.

You might also need to calculate the **range** (how spread out the data is). To do this find the largest data value and subtract the smallest data value from it. You shouldn't include anomalous results when calculating the mean or the range.

Tip: When people talk about an average, they are usually referring to the mean value.

Example — Maths Skills

Compare the mean absorbance and range of absorbance for test tubes A and B in the table on the right.

| Test tube | Absorbance of solution / absorbance units (AU) | | |
|-----------|--|----------|----------|
| | Repeat 1 | Repeat 2 | Repeat 3 |
| A | 0.31 | 0.50 | 0.52 |
| B | 1.2 | 0.84 | 1.5 |

To calculate the means:

- Add up the three data values for A, then divide by three.
A: $(0.31 + 0.50 + 0.52) \div 3 = 1.33 \div 3 = 0.44 \text{ AU}$ (2 s.f.)
- Do the same for B.
B: $(1.2 + 0.84 + 1.5) \div 3 = 3.54 \div 3 = 1.2 \text{ AU}$ (2 s.f.)
B has the higher mean.

Tip: S.f. stands for 'significant figures'. You can find out how many significant figures to use when rounding answers on page 7.

To find the range of results for each test tube, subtract the smallest result from the largest result.

$$\text{A: } 0.52 - 0.31 = 0.21 \text{ AU} \quad \text{B: } 1.5 - 0.84 = 0.66 \text{ AU}$$

A has the smaller range.

Tip: The mean (and other averages, see next page), range and standard deviation are all examples of descriptive statistics. Descriptive statistics simply describe any patterns in the data.

Standard deviation

Standard deviation can be more useful than the range because it tells you how values are spread about the mean rather than just the total spread of data. A small standard deviation means the repeated results are all similar and close to the mean, i.e. they are precise.

Tip: There's more on standard deviation on pages 249-251.

Median and mode

Like the mean, the median and mode are both types of average.

To calculate the median, put all your data in numerical order.

The median is the middle value in this list. If you have an even number of values, the median is halfway between the middle two values.

To calculate the mode, count how many times each value comes up.

The mode is the number that appears most often. A set of data might not have a mode — or it might have more than one.

Example — Maths Skills

The heights of bean plants (in millimetres) were measured six weeks after planting. The results were as follows:

112 102 106 120 98 106 80 105 106 110 95 98

Calculate the median and mode of these results.

1. Put the data in numerical order:

80 95 98 98 102 105 106 106 106 110 112 120

2. Find the middle value (the median):

There are 12 values, so the median is between the 6th and 7th numbers. The 6th number is 105 and the 7th is 106, so the median is 105.5 mm.

3. Count how many times each value comes up to find the mode:

106 comes up three times. None of the other numbers come up more than twice. So the mode is 106 mm.

Tip: If all the values in your data are different, there won't be a mode at all.

Tip: To find the value halfway between two numbers, add the two numbers together and then divide by two.
E.g. $105 + 106 = 211$, $211 \div 2 = 105.5$.

Calculating percentages

Calculating **percentages** helps you to compare amounts from samples of different sizes. To give the amount X as a percentage of sample Y, you need to divide X by Y, then multiply by 100.

Example — Maths Skills

In a DNA molecule containing 3000 bases, 900 of the bases are cytosine. What percentage of the bases are cytosine?

1. Divide 900 by 3000: $900 \div 3000 = 0.30$
2. Multiply by 100: $0.30 \times 100 = 30\%$

Calculating percentage change

Calculating **percentage change** helps to quantify how much something has changed, e.g. the percentage change in the growth rate of pea plants when a fertiliser is added. To calculate it you use this equation:

$$\text{Percentage change} = \frac{\text{final value} - \text{original value}}{\text{original value}} \times 100$$

A positive value indicates an increase and a negative value indicates a decrease.

Example — Maths Skills

A person's blood glucose concentration before a meal was 4.2 mmol dm^{-3} .

Two hours after a meal it was 6.5 mmol dm^{-3} .

Calculate the percentage change.

$$\text{Percentage change} = \frac{6.5 - 4.2}{4.2} \times 100 = 55\% \text{ (2 s.f.)}$$

Exam Tip

The examiners just love getting you to calculate percentage changes, including percentage increases and decreases, so make sure you learn this formula.

Tip: This means that the person's blood glucose concentration was 55% higher after the meal.

Percentage change can be either positive or negative, depending on whether the value has gone up or down. However, percentage increase and percentage decrease are both written as positive numbers because the direction of the change has already been taken into account.

Using ratios

Ratios can be used to compare lots of different types of quantities. For example, an organism with a surface area to volume ratio of 2 : 1 would theoretically have a surface area twice as large as its volume.

Ratios are usually most useful in their simplest (smallest) form. To simplify a ratio, divide each side by the same number. It's in its simplest form when there's nothing left you can divide by. To get a ratio of X : Y in the form X : 1, divide both sides by Y.

Examples — Maths Skills

- To simplify the ratio 28 : 36, divide both sides by 4. You get **7 : 9**.
- To write the ratio 28 : 36 in the form of X : 1, just divide both sides by 36:
 $28 \div 36 = 0.78$ $36 \div 36 = 1$
So the ratio is **0.78 : 1**.

Tip: If you're not sure what number to divide by to simplify a ratio, start by trying to divide both sides by a small number, e.g. 2 or 3, then check to see if you can simplify your answer further. E.g. you could simplify 28 : 36 by dividing each side by 2 to get 14 : 18. But you could simplify it further by dividing by 2 again to get 7 : 9. You can't simplify the ratio any further, so it's in its simplest form.

Rounding to significant figures

The first **significant figure** of a number is the first digit that isn't a zero. The second, third and fourth significant figures follow on immediately after the first (even if they're zeros). When you're processing your data you may well want to round any really long numbers to a certain number of significant figures.

Example

0.6874976 rounds to **0.69** to 2 s.f. and to **0.687** to 3 s.f.

When you're doing calculations using measurements given to a certain number of significant figures, you should give your answer to the lowest number of significant figures that was used in the calculation.

Example — Maths Skills

For the calculation: $1.2 \div 1.85 = 0.648648648\dots$

1.2 is given to 2 significant figures. 1.85 is given to 3 significant figures. So the answer should be given to 2 significant figures.

Round the final significant figure (0.648) up to 5: $1.2 \div 1.85 = \mathbf{0.65}$ (**2 s.f.**)

Tip: You may also want to round measurements to a certain number of significant figures when you're recording your data, e.g. if you're using a data logger that records data to several decimal places.

The lowest number of significant figures in the calculation is used because the fewer digits a measurement has, the less accurate it is. Your answer can only be as accurate as the least accurate measurement in the calculation.

Tip: When rounding a number, if the next digit after the last significant figure you're using is less than 5 you should round it down, and if it's 5 or more you should round it up.

Writing numbers in standard form

When you're processing data you might also want to change very big or very small numbers that have lots of zeros into something more manageable — this is called standard form.

Examples

1 000 000 can be written 1×10^6 . 0.017 can be written 1.7×10^{-2} .

To do this you just need to move the decimal point left or right. The number of places the decimal point moves is then represented by a power of 10 — this is positive for big numbers, and negative for numbers smaller than one.

Tip: When you're writing a measurement in standard form, make sure you keep the same number of significant figures. E.g. $0.00400 \text{ cm}^3 = 4.00 \times 10^{-3} \text{ cm}^3$. This'll make sure that you don't lose any accuracy.

Tip: Double check you've got it right by doing the multiplication — you should end up with the number you started with. So for this example, you'd check $1.65 \times 10^4 = 16\,500$.

Tip: One decimetre cubed (1 dm^3) is the same as one litre (1 L).

Tip: Make sure your answer makes sense — if you're converting from a small unit (e.g. cm^3) to a larger unit (e.g. dm^3) you need to divide the value, so your answer should be smaller than the number you started with.

Tip: Statistical tests are inferential statistics. This means they allow you to 'infer' things (draw conclusions) from the data.

Example — Maths Skills

To write 16 500 in standard form:

- Move the decimal point to give the smallest number you can between 1 and 10.

$$\begin{array}{r} \text{16 500} \\ \longrightarrow \\ 1.6500 \end{array}$$

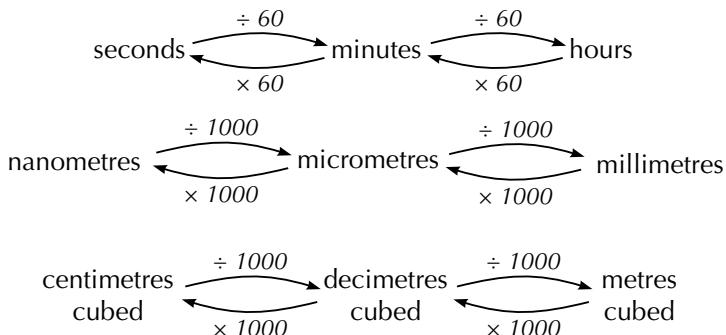
- Count the number of places the decimal point has moved.
The decimal point has moved four places to the left.
- Write that number as the power of ten. If the decimal point has moved to the left, the power is positive. If the decimal point has moved to the right, the power is negative.

$$16\,500 = 1.65 \times 10^4$$

Converting between units

When processing your data, you need to have all the data in the correct units. Make sure you can convert between common units of time, length and volume.

Examples



Examples — Maths Skills

- 10 cm³ of glucose solution is needed to create a dilution series. What is the volume of glucose solution needed in dm³?**

There are 1000 cm^3 in one dm^3 , so you need to divide by 1000:
 $10 \text{ cm}^3 \div 1000 = 0.01 \text{ dm}^3$

- The volume of carbon dioxide produced over time was measured in an experiment into aerobic respiration in yeast. The rate of CO₂ production was found to be $3.7 \text{ cm}^3 \text{ min}^{-1}$. What is this rate in $\text{cm}^3 \text{s}^{-1}$?**

$3.7 \text{ cm}^3 \text{ min}^{-1}$ means 3.7 cm^3 carbon dioxide was produced per minute. You want to find out the volume produced per second. There are 60 seconds in one minute, so you need to divide the volume by 60:
 $3.7 \text{ cm}^3 \text{ min}^{-1} \div 60 = 0.06 \text{ cm}^3 \text{ s}^{-1}$

Statistical tests

Statistical tests are used to analyse data mathematically. You can be more confident in your **conclusions** (see page 15), if they're based on results that have been analysed using a statistical test.

If you're planning on analysing your data using a statistical test, you first need to come up with a **null hypothesis** — this is a special type of hypothesis that states there is no significant difference (or correlation) between the things you're investigating. You then collect data to try to disprove the null hypothesis before analysing it statistically. There's more on null hypotheses on pages 257 and 393.

Student's t-test

You can use the Student's t-test when you have two sets of data that you want to compare. It tests whether there is a significant difference in the means of the two data sets. The value obtained is compared to a critical value, which helps you decide how likely it is that the results or 'differences in the means' were due to chance. If the value obtained from the t-test is greater than the critical value at a probability (**P value**) of 5% or less (≤ 0.05), then you can be 95% confident that the difference is significant and not due to chance. This is called a **95% confidence limit** — which is good enough for most biologists to reject the null hypothesis.

Chi-squared test

You can use the chi-squared test when you have categorical (grouped) data and you want to know whether your observed results are statistically different from your expected results. You compare your result to a critical value — if it's larger than the critical value at $P = 0.05$, you can be 95% certain the difference is significant. There's more on chi-squared on pages 393-395.

Correlation coefficient

A correlation coefficient allows you to work out the degree to which two sets of data are correlated (see page 15 for more on correlation). It is given as a value between 1 and -1. A value of 1 indicates a strong positive correlation, 0 means there is no correlation and -1 is a strong negative correlation. You can then compare your result to a critical value to find out whether or not the correlation is significant. The **Spearman's rank correlation coefficient** is an example of a correlation coefficient. See pages 256-257 for how to use it.

Exam Tip

In the exams, you could be asked which statistical test you'd use to analyse some data and to explain why you'd use it, as well as to interpret the test result.

Exam Tip

When you're talking about the results of a statistical test and using the 95% confidence limit, make sure you refer to the probability as less than 0.05 or 5%, not 0.05%.

Tip: If the result of your statistical test is greater than the critical value at a P value of less than 2% (< 0.02), or even 1%, you can be even more confident that the difference is significant.

Tip: Make sure you're familiar with the symbols $>$ (greater than), $>>$ (much greater than), $<$ (less than) and $<<$ (much less than).

4. Presenting Data

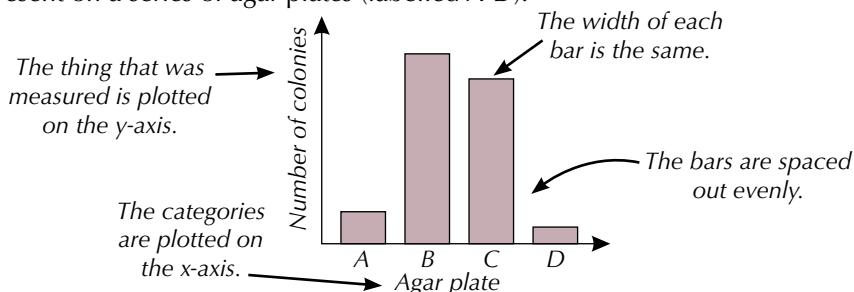
Presenting your data can make it easier for you to understand your results and spot any trends. You need to choose the best way to present your data.

Qualitative and discrete data

Qualitative data is non-numerical data, e.g. blood group. **Discrete** data is numerical data that can only take certain values in a range, e.g. number of patients. You can use **bar charts** or **pie charts** to present these types of data.

Example

The bar chart below shows the number of bacterial colonies present on a series of agar plates (labelled A-D).



Tip: Qualitative data can also be called categorical data — all the data can be sorted into categories and values between categories don't exist.

Exam Tip

If you are asked to draw a graph or chart in your exam, don't forget to label the axes (including the quantity and units), choose a sensible scale and make sure that it covers at least half the graph paper.

Tip: The graph on the right is a line graph. Line graphs look a bit like scattergrams (see next page), but the points on line graphs are joined together.

Tip: Don't be fooled by the height of the bars in a histogram — the tallest bar doesn't always belong to the class with the greatest frequency.

Tip: The data for a histogram is split into groups called classes, rather than categories. The class width is the range of the class.

Tip: The continuous data here has been split into classes. $0 \leq x < 5$ means the data in the class is more than or equal to 0 and less than 5.

Tip: If all the class widths are the same, you can just plot the frequency on the y-axis.

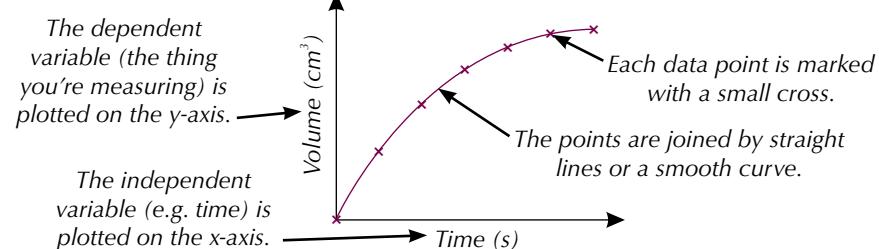
Continuous data

Continuous data is data that can take any value in a range, e.g. height or weight. You can use **line graphs** or **histograms** to present this type of data.

Line graphs

Line graphs often show how a variable changes over time. The data on both axes is continuous.

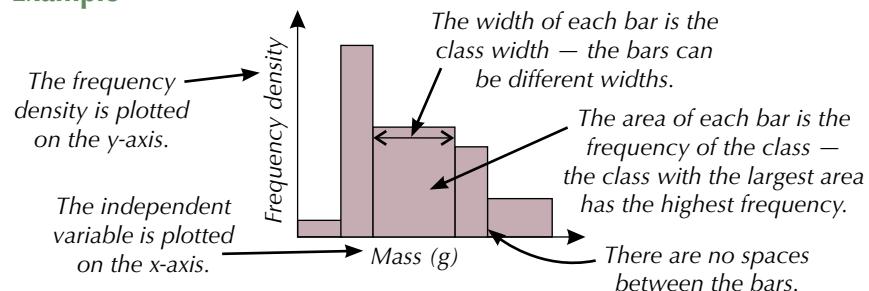
Example



Histograms

Histograms are a useful way of displaying frequency data when the independent variable is continuous. They may look like bar charts, but it's the area of the bars that represents the frequency (rather than the height). The height of each bar is called the **frequency density**.

Example



You calculate the frequency density using this formula:

$$\text{frequency density} = \text{frequency} \div \text{class width}$$

Example — Maths Skills

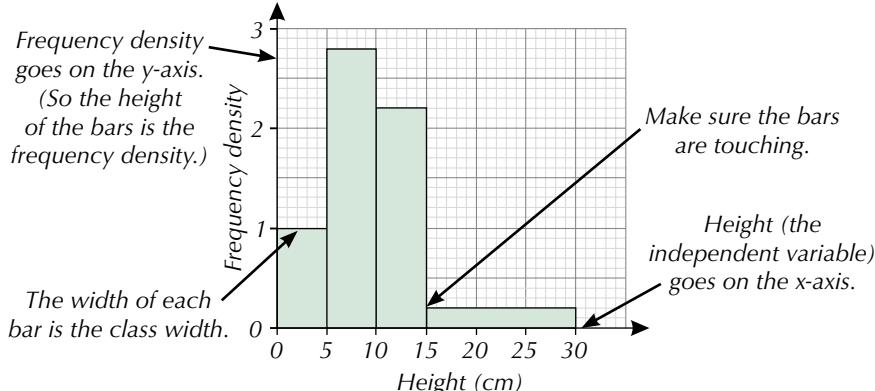
The table on the right shows the results of a study into variation in pea plant height. The heights of the plants were grouped into four classes.

| Height of pea plant (cm) | Frequency |
|--------------------------|-----------|
| $0 \leq x < 5$ | 5 |
| $5 \leq x < 10$ | 14 |
| $10 \leq x < 15$ | 11 |
| $15 \leq x < 30$ | 3 |

- To draw a histogram of the data, you first need to work out the width of each class. Write the class width in a new column.

| Class width |
|----------------|
| $5 - 0 = 5$ |
| $10 - 5 = 5$ |
| $15 - 10 = 5$ |
| $30 - 15 = 15$ |

- Use the formula on the previous page to calculate the frequency density for each class and write it in another new column.
- Work out a suitable scale for each axis, then plot the histogram.
It should look something like this:



Tip: You might have to round the frequency density — if so, choose a sensible number of decimal places that will be possible to plot using your graph's scale.

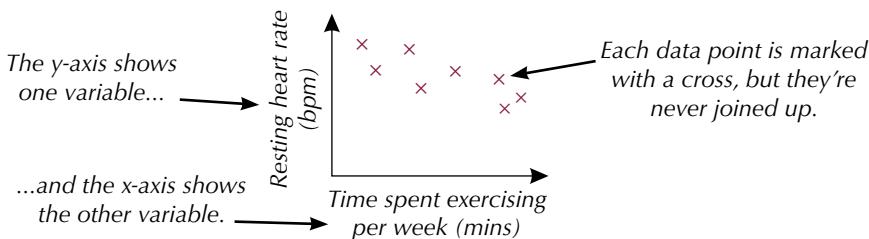
Tip: You can calculate the frequency of a class from a histogram by rearranging the formula: frequency = frequency density \times class width.

Tip: The width of the whole histogram shows the range of results (how spread out they are).

Scattergrams

When you want to show how two variables are related (or correlated, see page 15) you can use a **scattergram**. Both variables must be numbers.

Example



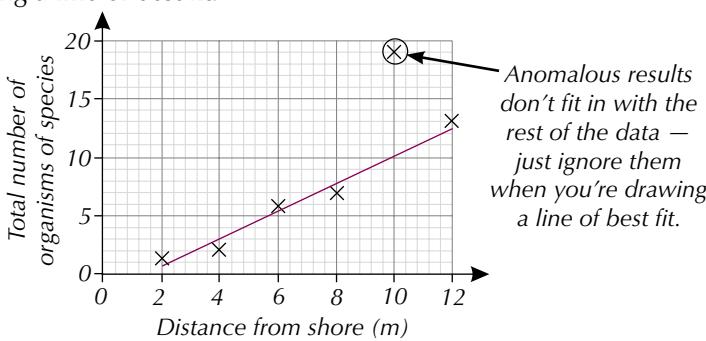
Tip: Scattergrams can also be called scatter graphs, or scatter diagrams.

Tip: Data that's made up of numbers is called quantitative data.

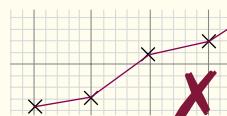
You can draw a **line** (or curve) **of best fit** on a scattergram to help show the trend in your results. To do so, draw the line through or as near to as many points as possible, ignoring any anomalous results.

Example

The number of organisms of one species on a rocky beach was recorded at different distances from the shore. The graph below shows the results, including a line of best fit.



Tip: You should never join the points together on a scattergram.



A line of best fit shows a trend rather than changes between data points.

Tip: A trend shown by a scattergram is called a **correlation**. There's more about correlation and what it means on page 15.

Finding the rate from a graph

Rate is a measure of how much something is changing over time.

Calculating a rate can be useful when analysing your data, e.g. you might want to find the rate of a reaction. You can find the rate from a graph that shows a variable changing over time by finding the **gradient** (how steep it is):

Tip: Linear graphs are graphs with a straight line.

Tip: When using this equation to find a rate, x should always be the time.

Tip: The graph on the right is a linear graph in which one variable increases in proportion with the other. The symbol for 'proportional to' is ' \propto '. Here, you can say that volume of oxygen \propto time.

Tip: When drawing a triangle to calculate a gradient like this, the hypotenuse of the triangle should be at least half as long as the line of the graph itself.

Tip: The units for the gradient are the units for y divided by the units for x . Remember, $\text{cm}^3 \text{s}^{-1}$ means the same as cm^3/s (centimetres cubed per second).

Linear graphs

For a linear graph you can calculate the rate by finding the gradient of the line, using the equation:

$$\text{Gradient} = \frac{\text{Change in } y}{\text{Change in } x}$$

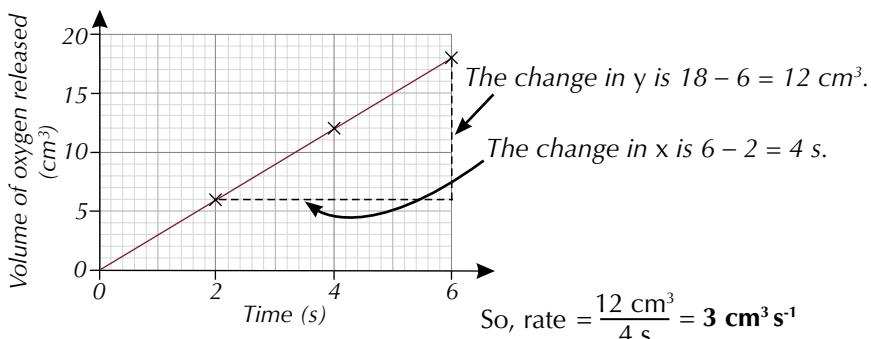
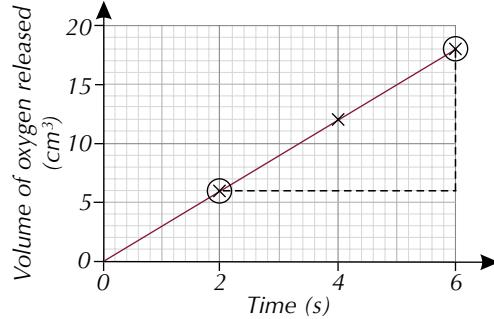
Change in y is the change in value on the y -axis and **change in x** is the change in value on the x -axis.

The equation of a straight line can always be written in the form $y = mx + c$, where m is the gradient and c is the y -intercept (this is the value of y when the line crosses the y -axis).

Example — Maths Skills

To find the rate at which oxygen is produced in the graph on the right:

1. Pick two points on the line that are easy to read and a good distance apart.
2. Draw a vertical line down from one point and a horizontal line across from the other to make a triangle.
3. Use the scales on the axes to work out the length of each line. The vertical side of the triangle is the change in y and the horizontal side of the triangle is the change in x .



To find the equation of the line you need the gradient (which is the same as the rate) and the y -intercept (where the line crosses the y -axis).

The gradient is 3 and the line crosses the y -axis where y is 0.

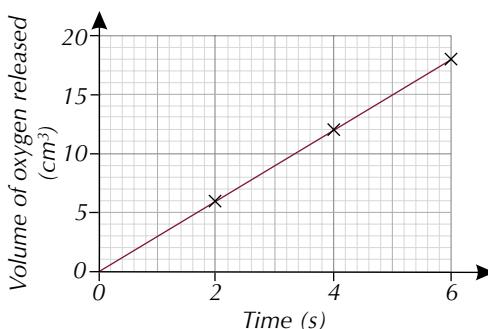
So the equation for the line is $y = 3x + 0$.

Since $c = 0$, the equation can be written as just $y = 3x$.

Knowing the equation of the line allows you to estimate results not plotted on the graph:

Example — Maths Skills

For the reaction shown in the graph on the right, estimate the volume of oxygen released after 20 seconds.



The equation for the line is $y = 3x$ (see previous page), where y is the volume of oxygen released (in cm^3) and x is the time (in seconds).

To find the value of y when x is 20 s, just replace x with 20 in the equation.

$$y = 3 \times 20 = 60 \text{ cm}^3$$

Tip: This is an estimate because you are assuming that the relationship between the two variables doesn't change after six seconds (so as time increases, the volume of oxygen released keeps increasing at the same rate).

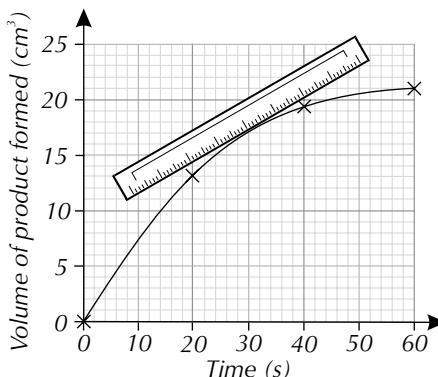
Curved graphs

For a curved (non-linear) graph you can find the rate by drawing a **tangent**. A tangent is a straight line that touches a single point on the curve.

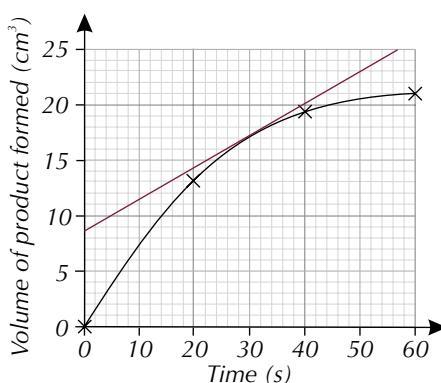
Example — Maths Skills

To find the rate of reaction when time = 30 seconds on the graph below:

1. Position a ruler on the graph at the point on the curve where you want to know the rate (so on this graph, find the point on the curve where $x = 30$).
2. Angle the ruler so there is equal space between the ruler and the curve on either side of the point.
3. Draw a line along the ruler to make the tangent. Extend the line right across the graph — it'll help to make your gradient calculation easier as you'll have more points to choose from.



Tip: Look at the gaps on either side of the point — keep wiggling the ruler about until the two gaps look about the same size.

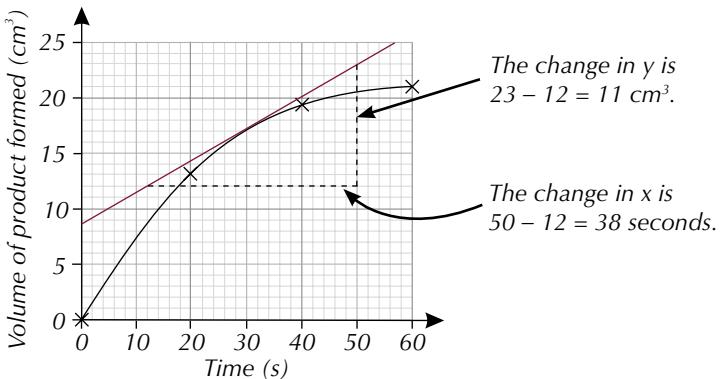


Tip: Always use a sharp pencil when drawing a tangent — you'll need to read points off the line in the next step, so make sure the line's nice and clear.

Tip: Remember,
gradient = change in $y \div$
change in x .

Tip: Remember, the
gradient of a tangent
only tells you the rate at
that particular point on
the graph.

4. To find the rate, calculate the gradient of the tangent in the same way you would calculate the gradient of a straight line graph (see p. 12).



$$\text{Gradient} = 11 \text{ cm}^3 \div 10 \text{ seconds} = 0.29 \text{ cm}^3 \text{ s}^{-1}$$

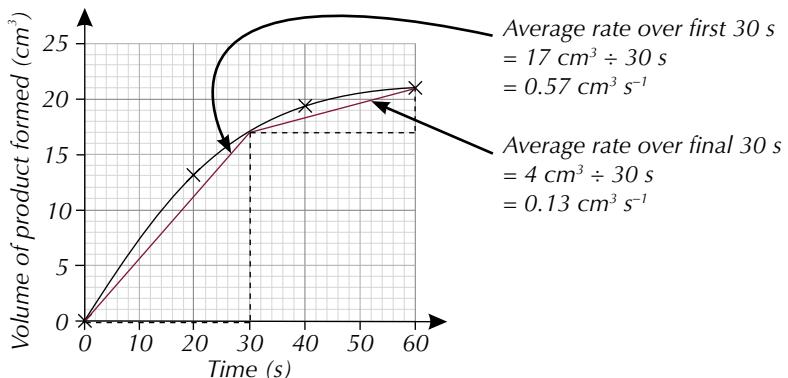
In the exam, you might be asked to give the ratio of the rates at different times in a reaction. Don't panic — just use the method above to find the rate at each time and give your answer as a ratio (see example below).

You might also be asked to find the average (or mean) rate over a particular period of time — if so, you can just draw a straight line between the first and last point in that time period and calculate the gradient of the line to find the rate. This is also shown in the example below.

Example — Maths Skills

Find the ratio of the average rate of reaction over the first 30 seconds to the average rate of reaction during the final 30 seconds on the graph below. Give your answer in the form X : 1.

Tip: There's more on ratios and converting them into the form $X : 1$ on page 7.



$$\text{Ratio} = 0.57 : 0.13$$

Divide both sides by 0.13 to get the ratio in the form $X : 1$.

$$\text{Ratio} = 4.4 : 1$$

5. Concluding and Evaluating

You need to be able to draw conclusions from your results and evaluate them. You also need to be able to draw conclusions from other people's data and evaluate them — which is what you're likely to be asked to do in your exams.

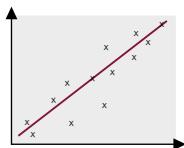
Drawing conclusions from data

Conclusions need to be **valid**. A conclusion can only be considered as valid if it uses valid data (see page 1).

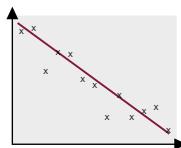
Correlations and causal relationships

You can often draw conclusions by looking at the relationship (**correlation**) between two variables:

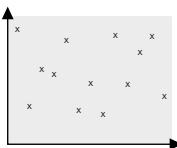
Positive
As one variable increases the other increases.



Negative
As one variable increases the other decreases.



No correlation
There is no relationship between the variables.



Tip: The closer the points are to the line of best fit, the stronger the correlation. You can calculate a correlation coefficient (see p. 9) to get a numerical value for how strong the correlation is.

You have to be very careful when drawing conclusions from data like this because a correlation between two variables doesn't always mean that a change in one variable causes a change in the other (the correlation could be due to chance or there could be a third variable having an effect).

If there's a relationship between two variables and a change in one variable does cause a change in the other it's called a **causal relationship**. It can be concluded that a correlation is a causal relationship if every other variable that could possibly affect the result is controlled.

Tip: In reality, concluding that a correlation is a causal relationship is very hard to do — correlations are generally accepted to be causal relationships if lots of studies have found the same thing, and scientists have figured out exactly how one factor causes the other.

Drawing specific conclusions

When you're making a conclusion you can't make broad generalisations from data — you have to be very specific. You can only conclude what the results show and no more.

Example — Maths Skills

Figure 1 shows the results from an investigation into the effect of concentration of a plant growth factor X on the height of Plant Species A.

What you can conclude from these results:

The only conclusion you can draw is that as the concentration of growth factor X increases, the height of Plant Species A increases.

What you can't conclude from these results:

You can't conclude that this is true for any other plant growth factor or any other plant species — the results could be completely different. Without more information about what other variables were controlled, you can't conclude that the increasing concentration of growth factor X has caused the increase in height of Plant Species A either.

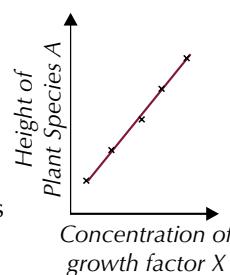


Figure 1: The relationship between concentration of growth factor X and height of Plant Species A.

Exam Tip

Being able to recognise correlations and causal relationships comes up a lot in Biology. It's really important that you learn how to do this and understand the difference between the two.

Tip: A reading is when you make a judgement about one value, e.g. when you read a value off a mass balance. A measurement is when you judge two values and find the difference, e.g. when you measure length with a ruler.

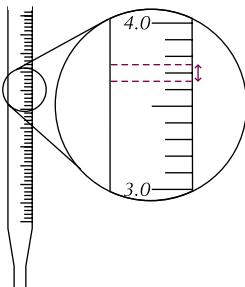


Figure 2: The margin of error for a reading of 3.7 cm^3 using a 10 cm^3 pipette.

Tip: You usually quote an uncertainty to the same number of decimal places as the value — so we've rounded 0.25 up to 0.3 here.

Uncertainty in data

When you draw a conclusion, it's often a good idea to talk about the uncertainty in your data — in other words, the amount of error there might be. The results you get from an experiment won't be completely perfect — there'll always be a degree of uncertainty in your readings or measurements due to limits in the sensitivity of the apparatus you're using.

A \pm sign tells you the range in which the true value lies (usually to within a 95% confidence level). The range is called the **margin of error**.

Example

A 10 cm^3 pipette has graduations to mark every 0.1 cm^3 . If you measure a volume with it, you are measuring to the nearest 0.1 cm^3 — the real volume could be up to 0.05 cm^3 less or 0.05 cm^3 more. The uncertainty value of the pipette is $\pm 0.05\text{ cm}^3$, and so its margin of error is 0.1 cm^3 (see Figure 2).

If you're combining readings or measurements, you'll need to combine their uncertainties:

Example — Maths Skills

In a serial dilution, 5.0 cm^3 of glucose solution is transferred using a pipette that measures to the nearest 0.5 cm^3 . It is added to 10.0 cm^3 water that was measured in a graduated cylinder with graduations to mark every 1.0 cm^3 .

The uncertainty in the pipette is $\pm 0.25\text{ cm}^3$, or 0.3 cm^3 to 1 d.p.
The uncertainty in the graduated cylinder is $\pm 0.5\text{ cm}^3$.

So the total uncertainty will be $0.3\text{ cm}^3 + 0.5\text{ cm}^3 = \pm 0.8\text{ cm}^3$.

Calculating percentage error

If you know the uncertainty value of your measurements, you can calculate the percentage error using:

$$\text{percentage error} = \frac{\text{uncertainty}}{\text{reading}} \times 100$$

Example — Maths Skills

50 cm^3 of HCl is measured with an uncertainty value of $\pm 0.05\text{ cm}^3$.

The percentage error = $\frac{0.05}{50} \times 100 = 0.1\%$

Minimising errors in data

One obvious way to reduce errors in your measurements is to buy the most sensitive equipment available. In real life there's not much you can do about this one — you're stuck with whatever your school or college has got. But there are other ways to lower the uncertainty in experiments.

Example — Measuring a greater amount of something

Using a 500 cm^3 cylinder with an uncertainty value of $\pm 2.5\text{ cm}^3$ to measure 100 cm^3 of liquid will give you a percentage error of: $\frac{2.5}{100} \times 100 = 2.5\%$

But if you measure 200 cm^3 in the same cylinder, the percentage error is: $\frac{2.5}{200} \times 100 = 1.25\%$

Hey presto — you've just halved the uncertainty.

Tip: You can also minimise errors by using a larger sample size, as this reduces the chance of getting a freak result — see page 3.

Evaluating results

When you evaluate your results, you need to think about whether they were repeatable and reproducible and whether they were valid.

Repeatability

- Did you take enough repeat readings or measurements?
- Would you do more repeats if you were to do the experiment again?
- Did you get similar data each time you carried out a repeat measurement?

If you didn't do any repeats, or enough repeats, you can't be sure your data is repeatable. Your repeated results need to be similar too. If you repeated a measurement three times and got a completely different result each time, your results aren't repeatable (or precise).

Tip: Think about whether other scientists could gain data showing the same relationships that are shown in your data.

Reproducibility

Have you compared your results with other people's results and if so, were they similar? If not, you can't be sure your data is reproducible.

Validity

- Does your data answer the question you set out to investigate?
- Were all the variables controlled?

If you didn't control all the variables, you haven't answered the original question and your data isn't valid.

Exam Tip
If you're given data or a method to evaluate in the exam, you should be asking similar questions, e.g. were all the variables controlled? And if not, how should they have been controlled?

Example

You could only conclude from your results that the rate of photosynthesis increases with light intensity (see page 1) if you controlled all the other variables that could have affected rate of photosynthesis in your experiment, e.g. pH, temperature, etc.

Evaluating methods

When you evaluate your method, you need to think about how you could improve your experiment if you did it again. Here are some things to consider:

- Is there anything you could have done to make your results more precise or accurate?
- Were there any limitations in your method, e.g. should you have taken measurements more frequently?
- Was your sample size large enough?
- Were there any sources of error in your experiment?
- Could you have used more sensitive apparatus or equipment?

Tip: This is where you take the uncertainty of your measurements (see previous page) into account. Think about the size of the margin of error, and whether you could have reduced the uncertainty.

Having confidence in your conclusion

Once you've evaluated your results and method, you can decide how much confidence you have in your conclusion. For example, if your results are repeatable, reproducible and valid and they back up your conclusion then you can have a high degree of confidence in your conclusion.

You can also consider these points if you're asked to evaluate a conclusion in the exam.

Exam Tip

Data questions are fairly common in the exams. You might be given a conclusion for the data and asked to evaluate it — this just means you have to give reasons why it is (or isn't) a valid conclusion. You could also be asked how far data supports a conclusion — it requires a similar type of answer.

Example

A study examined the effect of soil pH on marram grass. A 40 m transect was set up, running from the shoreline and heading inland. The percentage cover of marram grass was measured by placing a 1 m² quadrat (divided into 100 squares) at 5 m intervals along the transect and counting the number of squares containing marram grass. At each sample point, the pH of the soil was measured using a pH probe. The results are shown in Figure 3:

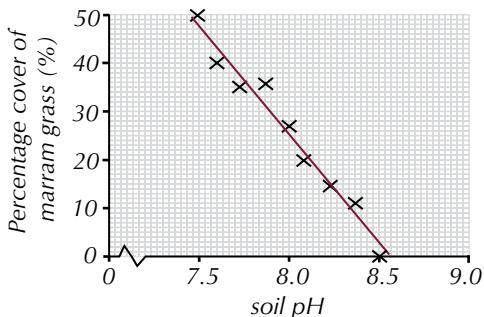


Figure 3: Scattergram to show relationship between soil pH and percentage cover of marram grass.

A student who read this study concluded that in seashore habitats a higher soil pH causes the percentage cover of marram grass to decrease. Does the data support this conclusion? Explain your answer.

Yes — The data in the graph supports the conclusion as it shows that as the soil pH increases, the percentage cover of marram grass decreases — soil pH has a negative correlation with the percentage cover of marram grass.

No — You can't conclude that increasing soil pH causes the percentage cover of marram grass to decrease. Other factors may have been involved — for example, the soil moisture content or salinity may have changed over the course of the transect, or there may have been more competition from other species. This means you don't know how valid the study is — you can't be sure that the factor being investigated (soil pH) is the only one affecting the thing being measured (percentage cover of marram grass).

Also, the study is quite small — only one 40 metre transect was used. The trend shown by the data may not appear if more than one transect was studied, or if a longer transect was used.

The results are also limited by the method of sampling. We don't know how the transect line was chosen. It could have been chosen in an area which had a higher percentage cover of marram grass than other places along the shoreline.

Overall — The limits of the study mean that the student's conclusion isn't well supported.

6. The Practical Endorsement

Alongside your A-level exams, you have to do a separate 'Practical Endorsement'. This assesses practical skills that can't be tested in a written exam.

What is the Practical Endorsement?

The Practical Endorsement is assessed slightly differently to the rest of your course. Unlike the exams, you don't get a mark for the Practical Endorsement — you just have to get a pass grade.

In order to pass the Practical Endorsement, you have to carry out at least twelve practical experiments and demonstrate that you can:

- use a range of specified apparatus, e.g. you must be able to use a colorimeter (see page 100),
- carry out a range of specified practical techniques, e.g. you must be able to use sampling techniques in fieldwork (see pages 423-426).

The twelve practicals that you do are most likely to be the twelve Required Practicals that form part of the AQA A-level Biology course — these cover all the techniques you need to be able to demonstrate for your Practical Endorsement. You may carry out other practicals as well, or instead, which may also count towards your Practical Endorsement. You'll do the practicals in class, and your teacher will assess you as you're doing them.

You'll need to keep a record of all your assessed practical activities. Required Practicals 1 to 6 are all part of the Year 1 material (Topics 1-4). Required Practicals 7 to 12 are all part of the Year 2 material (Topics 5-8).

Exam Tip

You could also get asked questions about the Required Practicals in your written exams.

Tip: Throughout this book, examples of methods you could use for the Required Practicals are marked up with a big stamp, like this one:

REQUIRED PRACTICAL 9

Assessment of the Practical Endorsement

When assessing your practical work, your teacher will be checking that you're able to do five things:

1. Follow written methods and instructions

Make sure you read any instructions you are given fully before you start work and that you understand what you're about to do. This will help you to avoid missing out any important steps or using the wrong piece of apparatus.

2. Use apparatus and investigative methods correctly

You need to be able to demonstrate that you can use apparatus and carry out practical techniques correctly (see above), and that you can do so without too many reminders from your teacher. This means being able to carry out a procedure in the right order, as well as being able to fix problems when you come across them.

You'll also need to show that you can identify which variables need to be controlled in an experiment and how to control them (see page 1). If you can't easily control all the variables, you'll need to show that you can find a way around this, e.g. by using a negative control (see page 2). Finally, you'll need to demonstrate that you can decide which apparatus to use and what measurements you'll take with it to get the most accurate results (see page 3 for more).

Tip: You won't necessarily need to demonstrate each of these things every time you carry out a practical.

Tip: You'll be given the opportunity to build up to some of the more difficult skills.

Tip: The CLEAPSS® website has a database with details of the potential harm that hazardous substances you're likely to come across could cause. It also has student safety sheets, and your school or college may have CLEAPSS® Hazcards® you can use. These are all good sources of information if you're writing a risk assessment.

Tip: Reporting your results may involve presenting them in a graph or chart. See pages 9-11 for more.

Tip: Your teacher might ask you to keep a lab book — a notebook in which you write up all your practical activities and record all the results.

Tip: If you're unsure whether the information on a website is true or not, try and find the same piece of information in a different place. The more sources you can find for the information, the more likely it is to be correct.

Tip: There are lots of slightly different ways of referencing sources, but the important thing is that it's clear where you found the information.

3. Use apparatus and materials safely

This means being able to carry out a risk assessment (see page 3) to identify the dangers in your experiment and what you can do to reduce the risks associated with those dangers. You'll also have to show that you can work safely both in the lab and in the field, using appropriate safety equipment to reduce the risks you've identified, and that you can adjust your method as you go along to make it safer if necessary.

4. Make observations and record results

You need to show that you can make accurate observations that are relevant to your experiment and that you can collect data that's valid, accurate and precise. When you record the data, e.g. in a table, you need to make sure you do so to an appropriate level of accuracy and you include things like the units. Make sure you follow the rules on drawing tables from page 4.

5. Carry out supporting research and write reports using appropriate references

You need to show that you can write up an investigation properly. As well as reporting your results and analysing them, you'll need to draw conclusions about your findings, describe the method and apparatus you used, and write down any safety precautions you took.

You'll also need to show that you can use computer software to process your data. This might mean drawing a graph of your results using the computer, or using a computer programme to carry out a statistical test.

You'll need to write up any research you've done too (e.g. to help you with planning a method or to draw your conclusions) and properly cite the sources that you've used.

Research, references and citations

You can use books or the Internet to carry out research, but there a few things you'll need to bear in mind:

- Not all the information you find on the Internet will be true. It's hard to know where information comes from on forums, blogs and websites that can be edited by the general public, so you should avoid using these. Websites of organisations such as the Nuffield Foundation and the National Health Service (NHS) provide lots of information that comes from reliable scientific sources. Scientific papers and textbooks are also good sources of reliable information.
- It may sound obvious, but when you're using the information that you've found during your research, you can't just copy it down word for word. Any data you're looking up should be copied accurately, but you should rewrite everything else in your own words.
- When you've used information from a source, you need to cite the reference properly. Citations allow someone else to go back and find the source of your information. This means they can check your information and see you're not making things up out of thin air. Citations also mean you've properly credited other people's data that you've used in your work. A citation for a particular piece of information may include the title of the book, paper or website where you found the information, the author and/or the publisher of the document and the date the document was published.

Topic 1 A: Biological Molecules

1. Molecules of Life

There are loads of different types of biological molecules that make up all cells and organisms, such as carbohydrates, amino acids, proteins and lipids, etc. This topic (and Topic 1B) is all about these biological molecules...

Evidence for evolution

Evidence for evolution is information that supports the **theory of evolution** — the theory that all organisms on Earth are descended from one or a few common ancestors and that they have changed and diversified over time.

There is, and has been, a huge variety of different organisms on Earth but they all share some biochemistry. They all contain the same groups of carbon-based compounds that interact in similar ways — for example, they use the same nucleic acids (DNA and RNA) as genetic material and the same amino acids to build proteins.

These similarities suggest that animals and plants have a common ancestor, which provides indirect evidence for evolution.

Monomers and polymers

Most carbohydrates, proteins and nucleic acids are **polymers**. Polymers are large, complex molecules composed of long chains of **monomers** joined together.

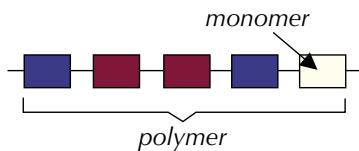


Figure 1: A polymer.

Monomers are small, basic molecular units that can form a polymer.

Examples of monomers include monosaccharides, amino acids and nucleotides.

Making polymers

Most biological polymers are formed from their monomers by **condensation** reactions. A condensation reaction forms a chemical bond between monomers, releasing a molecule of water — see Figure 2.

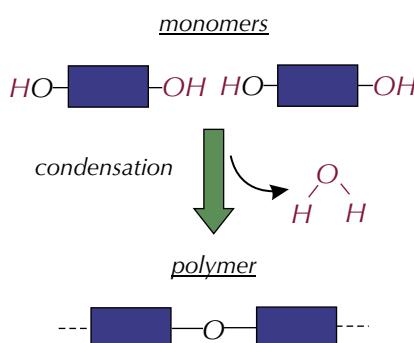


Figure 2: An example of the formation of a polymer.

Learning Objectives:

- Understand that the variety of life, both past and present, is extensive, but the biochemical basis of life is similar for all living things.
- Know that polymers are molecules made from a large number of monomers joined together.
- Know that monomers are the smaller units from which larger molecules are made.
- Know that monosaccharides, amino acids and nucleotides are examples of monomers.
- Understand that a condensation reaction joins two molecules together with the formation of a chemical bond and the elimination of a molecule of water.
- Understand that a hydrolysis reaction breaks a chemical bond between two molecules and involves a molecule of water.

Specification Reference 3.1.1

Exam Tip

If you're asked to show a condensation reaction, don't forget to put the water molecule in as a product.

Breaking down polymers

Tip: A condensation reaction removes one molecule of water, but a hydrolysis reaction adds a molecule of water.

Tip: It's easy to remember what a hydrolysis reaction does as 'hydro' means water and 'lysis' means breaking down.

Biological polymers can be broken down into monomers by **hydrolysis** reactions. A hydrolysis reaction breaks the chemical bond between monomers using a water molecule. It's basically the opposite of a condensation reaction.

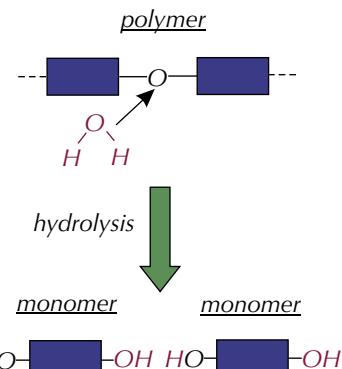


Figure 3: Hydrolysis of a polymer.

Practice Question — Application

- Q1 Cytochrome c is a protein used in the reactions of respiration and is found across species of animals, plants and unicellular organisms. Suggest why the widespread occurrence of cytochrome c is considered to be evidence for evolution.

Practice Questions — Fact Recall

- Q1 What is a polymer?
- Q2 What is a monomer?
- Q3 Give two examples of monomers.
- Q4 Explain what happens in a condensation reaction between two monomers.
- Q5 What type of reaction involves the breakage of a chemical bond between two monomers using water?

2. Sugars

Sugar is a general term for monosaccharides and disaccharides. Monosaccharides are the simplest sugars, and are the building blocks of carbohydrates.

Monosaccharides

All carbohydrates contain the elements C, H and O. The monomers that carbohydrates are made from are **monosaccharides**, e.g. glucose, fructose and galactose.

Glucose is a hexose sugar — a monosaccharide with six carbon atoms in each molecule. There are two types of glucose, alpha (α) and beta (β) glucose — they're isomers (molecules with the same molecular formula as each other, but with the atoms connected in a different way).

You need to learn the structures of both types of glucose for your exams — see Figure 1.

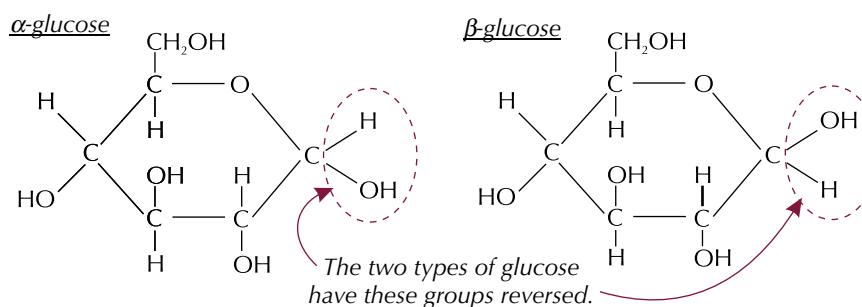


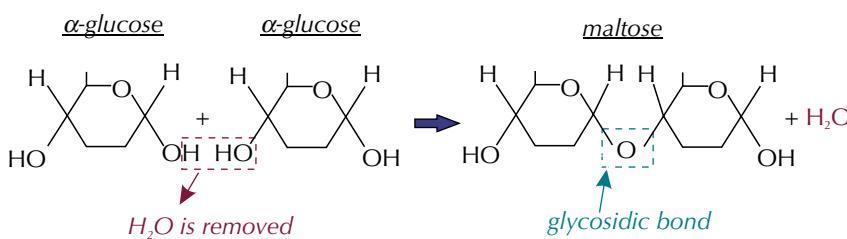
Figure 1: Glucose isomers.

Disaccharide formation

A disaccharide is formed when two monosaccharides join together. Monosaccharides are joined together by **condensation** reactions (see page 21) — a **glycosidic bond** forms between the two monosaccharides as a molecule of water is released.

Example

Two α -glucose molecules are joined together by a glycosidic bond to form maltose.



Sucrose is a disaccharide formed from a condensation reaction between a glucose molecule and a fructose molecule. Lactose is another disaccharide, formed from a glucose molecule and a galactose molecule.

Learning Objectives:

- Understand that monosaccharides are the monomers from which larger carbohydrates are made.
- Know that glucose, galactose and fructose are common monosaccharides.
- Know that glucose has two isomers, α -glucose and β -glucose, and recall their structures.
- Know that a condensation reaction between two monosaccharides forms a glycosidic bond.
- Understand that disaccharides are formed from two monosaccharides by condensation reactions:
 - maltose is a disaccharide formed by condensation of two glucose molecules,
 - sucrose is a disaccharide formed by condensation of a glucose molecule and a fructose molecule,
 - lactose is a disaccharide formed by condensation of a glucose molecule and a galactose molecule.
- Recall the Benedict's test for reducing and non-reducing sugars.

Specification Reference 3.1.2

Tip: You need to be aware of the hazards involved whenever you do practical work, including tests like these.

Tip: If the substance you want to test is a solid, you may have to prepare a solution of it before testing. You could do this by first crushing the solid with water and then filtering out the solid.

Tip: Always use an excess of Benedict's solution — this makes sure that all the sugar reacts.



Figure 3: A brick red colour indicates a positive Benedict's test result.



Figure 4: A blue colour indicates a negative Benedict's test result.

The Benedict's test for sugars

All sugars can be classified as **reducing sugars** or **non-reducing sugars**. To test for sugars you use the Benedict's test. The test differs depending on the type of sugar you're testing for.

Reducing sugars

Reducing sugars include all monosaccharides and some disaccharides, e.g. maltose and lactose. You add Benedict's reagent (which is blue) to a sample and heat it in a water bath that's been brought to the boil. If the test's positive it will form a coloured precipitate — solid particles suspended in the solution. The colour of the precipitate changes as shown in Figure 2.

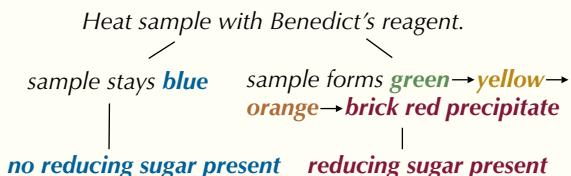


Figure 2: Benedict's test for reducing sugars.

The higher the concentration of reducing sugar, the further the colour change goes — you can use this to compare the amount of reducing sugar in different solutions. A more accurate way of doing this is to filter the solution and weigh the precipitate, or to remove the precipitate and use a **colorimeter** (see p. 100) to measure the absorbance of the remaining Benedict's reagent.

Non-reducing sugars

If the result of the reducing sugars test is negative, there could still be a non-reducing sugar present. To test for non-reducing sugars, like sucrose, first you have to break them down into monosaccharides. You do this by getting a new sample of the test solution (i.e. not the same one you've already added Benedict's reagent to), adding dilute hydrochloric acid and carefully heating it in a water bath that's been brought to the boil. Then you neutralise it by adding sodium hydrogencarbonate. Finally just carry out the Benedict's test as you would for a reducing sugar — see Figure 5.

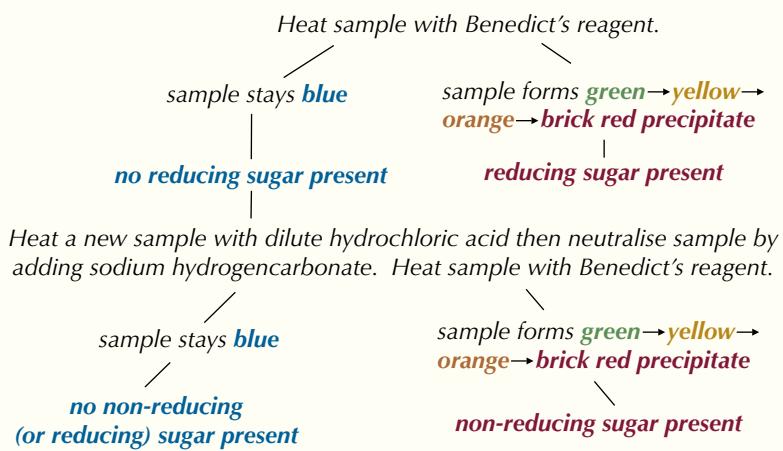
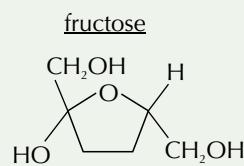
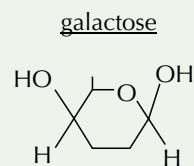
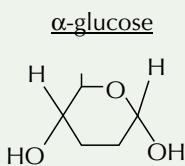


Figure 5: Benedict's test for non-reducing sugars.

Practice Questions — Application

Q1 Look at the following monosaccharides.



Draw the disaccharide that would be formed from a condensation reaction between:

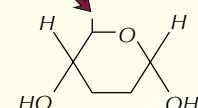
- a) α -glucose and galactose
- b) α -glucose and fructose

Q2 The table shows data from four different Benedict's tests.

What conclusions can you draw from each test?

| Test | Procedure | Result |
|------|---|--------|
| 1 | Sample heated with Benedict's reagent. | Blue |
| 2 | Sample heated with Benedict's reagent, (remained blue), then heated with hydrochloric acid and neutralised. Finally heated with Benedict's reagent. | Red |
| 3 | Sample heated with Benedict's reagent, (remained blue), then heat with hydrochloric acid and neutralised. Finally heated with Benedict's reagent. | Blue |
| 4 | Sample heated with Benedict's reagent. | Red |

Tip: Structures aren't always drawn with everything on them, e.g. when you get a line with nothing on the end, like this



it just means there's a carbon there, with other elements (like hydrogen) attached to it.

Tip: Remember, the samples are heated in a boiling water bath.

Practice Questions — Fact Recall

- Q1 Draw the structure of β -glucose.
- Q2 What is the name of the bond that forms between two monosaccharides?
- Q3 What molecule is released during a condensation reaction between two monosaccharides?
- Q4 Which monosaccharides make up the disaccharides:
 - a) maltose?
 - b) sucrose?
 - c) lactose?
- Q5 Describe how to test for reducing sugars and say what a positive and a negative result would look like.

Learning Objectives:

- Understand that polysaccharides can be formed by the condensation of many glucose units.
- Know that glycogen and starch are formed by the condensation of α -glucose.
- Know that cellulose is formed by the condensation of β -glucose.
- Recall the basic structure and functions of glycogen, starch and cellulose and the relationship of structure to function of these substances in animal cells and plant cells.

Specification Reference 3.1.2

Exam Tip

All you need to remember for questions like Q2 is to split them at the glycosidic bond and add a 'H' to either side.

3. Polysaccharides

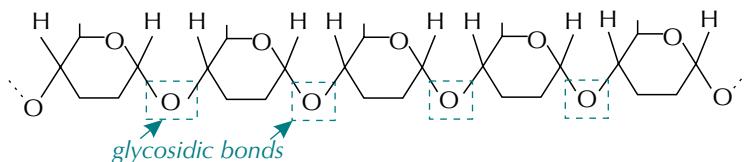
Polysaccharides are carbohydrates. Polysaccharide molecules are made from large numbers of their monomers (monosaccharides).

Polysaccharide formation and break down

A **polysaccharide** is formed when more than two monosaccharides are joined together by condensation reactions.

Example

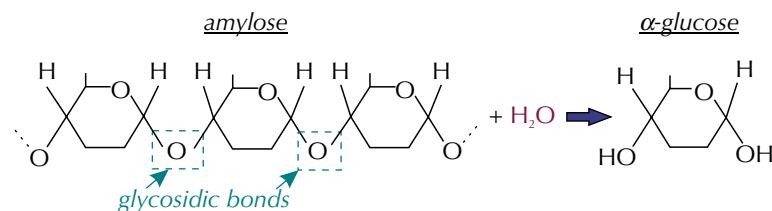
Lots of α -glucose molecules are joined together by glycosidic bonds to form amylose.



Polysaccharides can be broken down into their constituent monosaccharides by hydrolysis reactions.

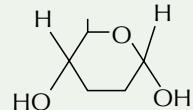
Example

Amylose is hydrolysed into α -glucose molecules.

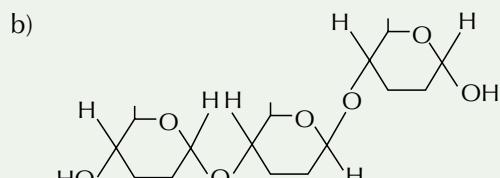
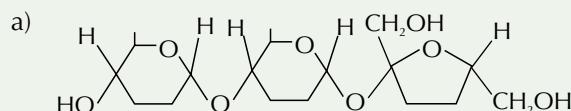


Practice Questions — Application

Q1 The structure of α -glucose is shown on the right. Draw a polysaccharide made of three α -glucose molecules.



Q2 Draw the monosaccharides produced from hydrolysis of the polysaccharides shown below.



Functions of polysaccharides

You need to know about the relationship between the structure and function of three polysaccharides — starch, glycogen and cellulose.

Starch

Cells get energy from glucose. Plants store excess glucose as starch (when a plant needs more glucose for energy, it breaks down starch to release the glucose). Starch is a mixture of two polysaccharides of alpha-glucose — amylose and amylopectin:

- **Amylose** is a long, unbranched chain of α -glucose. The angles of the glycosidic bonds give it a coiled structure, almost like a cylinder. This makes it compact, so it's really good for storage because you can fit more in to a small space.
- **Amylopectin** is a long, branched chain of α -glucose. Its side branches allow the enzymes that break down the molecule to get at the glycosidic bonds easily. This means that the glucose can be released quickly.

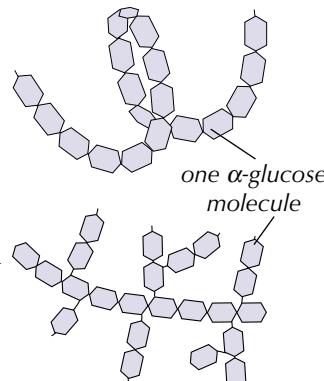


Figure 1: The structures of amylose (top) and amylopectin (bottom).

Starch is insoluble in water and doesn't affect water potential (see page 106), so it doesn't cause water to enter cells by osmosis, which would make them swell. This makes it good for storage.

Tip: You can test for the presence of starch using the iodine test (see next page).

Tip: Hydrogen bonds between α -glucose molecules help to hold amylose in its helical structure.

Exam Tip

When you're describing the structure of these polysaccharides, always specify whether you're talking about α -glucose or β -glucose — you won't get a mark for only saying glucose.

Glycogen

Animal cells get energy from glucose too. But animals store excess glucose as glycogen — another polysaccharide of alpha-glucose. Its structure is very similar to amylopectin, except that it has loads more side branches coming off it — see Figure 2. Loads of branches means that stored glucose can be released quickly, which is important for energy release in animals. It's also a very compact molecule, so it's good for storage.

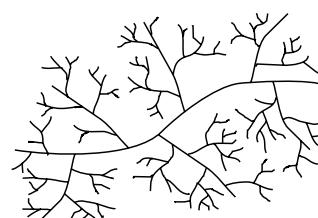


Figure 2: The structure of glycogen.

Tip: Starch is a large molecule, so it can't leave the cell — this is another reason why it's a good storage molecule.

Exam Tip

If you're asked about the function of glycogen in the exam, make sure you say it acts as an energy store or reserve — you won't get marks just for saying it 'contains energy'.

Cellulose

Cellulose is made of long, unbranched chains of beta-glucose. When beta-glucose molecules bond, they form straight cellulose chains. The cellulose chains are linked together by **hydrogen bonds** to form strong fibres called **microfibrils** — see Figure 3. The strong fibres mean cellulose provides structural support for cells (e.g. in plant cell walls).

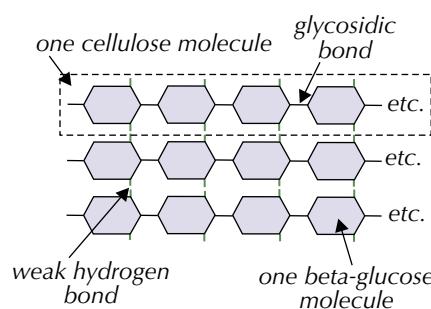


Figure 3: The structure of a cellulose microfibril.



Figure 4: Coloured scanning electron micrograph (SEM) of cellulose microfibrils in a plant cell wall.

The iodine test for starch

Exam Tip

Make sure you always talk about iodine in potassium iodide solution, not just iodine.



Figure 6: A dark blue-black colour indicates the presence of starch in an iodine test.

If you want to test for the presence of starch in a sample, you'll need to do the iodine test.

Just add iodine dissolved in potassium iodide solution to the test sample. If there is starch present, the sample changes from browny-orange to a dark, blue-black colour — see Figure 5.

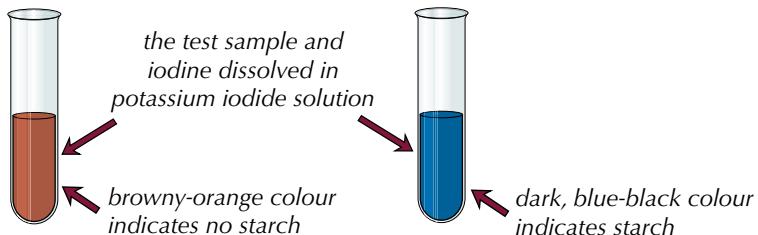


Figure 5: A negative (left) and positive (right) iodine test result.

Practice Questions — Fact Recall

- Q1 Name the type of monomer that makes up polysaccharides.
- Q2 What is the main energy storage material in:
- plants,
 - animals?
- Q3 a) Is starch soluble or insoluble?
b) Use your answer to a) to describe why starch is good for storage.
- Q4 a) Name the structures shown below:
- A
B
b) Explain an advantage of structure A that makes it suitable for energy storage.
- Q5 a) Which polysaccharide is the major component of plant cell walls?
b) Describe the structure of this polysaccharide, and explain how its structure makes it suited to its function in cell walls.
- Q6 Sketch and label a diagram of a microfibril.
- Q7 Describe the method you would use to test for the presence of starch, and say what a positive and a negative result would look like.

Exam Tip

Don't panic if you're asked to draw a diagram in the exam — you don't have to be the best artist in the world, but make sure you add labels to point out all the important bits.

4. Lipids

Lipids are commonly known as fats or oils. They're found all cells, and have a variety of different properties.

What are lipids made from?

Lipids are different from proteins (see pages 33-35) and carbohydrates (see pages 26-27) because they're not polymers formed from long chains of monomers. Lipids are made from a variety of different components, but they all contain **hydrocarbons** (molecules that contain only hydrogen and carbon atoms). The components they're made from relates to the lipid's function. There are two types of lipid you need to know about — triglycerides and phospholipids.

Triglycerides

Triglycerides have one molecule of glycerol with three fatty acids attached to it. Fatty acid molecules have long 'tails' made of hydrocarbons. The tails are 'hydrophobic' (they repel water molecules). These tails make lipids insoluble in water.

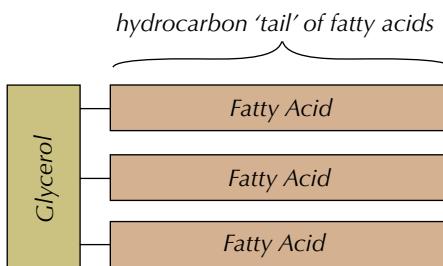


Figure 1: Structure of a triglyceride.

Fatty acids

All fatty acids consist of the same basic structure, but the hydrocarbon tail varies — see Figure 2. There are two kinds of fatty acids — saturated and unsaturated. The difference is in their hydrocarbon tails (R groups):

- Saturated fatty acids don't have any double bonds between their carbon atoms. The fatty acid is 'saturated' with hydrogen.

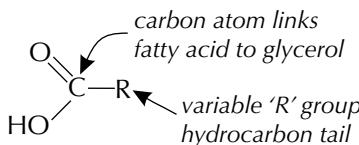


Figure 2: Structure of a fatty acid.

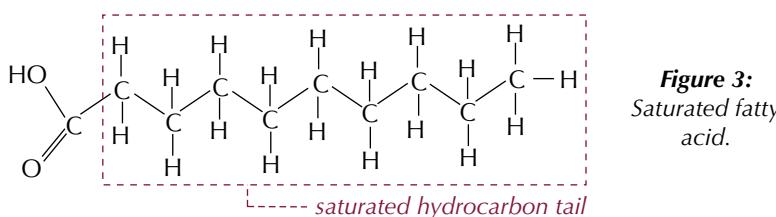


Figure 3:
Saturated fatty acid.

- Unsaturated fatty acids do have double bonds between carbon atoms, which cause the chain to kink.

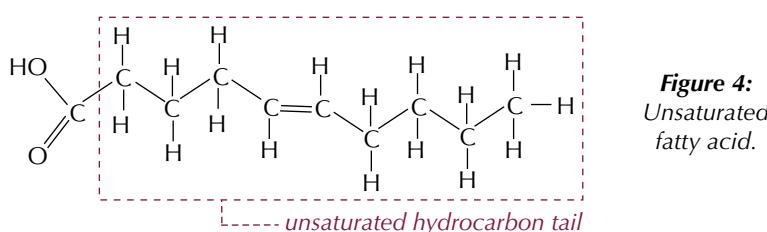


Figure 4:
Unsaturated fatty acid.

Learning Objectives:

- Know that triglycerides and phospholipids are two groups of lipid.
- Understand that triglycerides are formed by the condensation of one molecule of glycerol and three molecules of fatty acid.
- Know that the R-group of a fatty acid may be saturated or unsaturated.
- Be able to recognise, from diagrams, saturated and unsaturated fatty acids.
- Know that a condensation reaction between glycerol and a fatty acid (RCOOH) forms an ester bond.
- Understand that in phospholipids, one of the fatty acids of a triglyceride is substituted by a phosphate-containing group.
- Be able to explain the different properties of triglycerides and phospholipids.
- Know how the properties of triglycerides and phospholipids relate to their structures.
- Recall the emulsion test for lipids.

Specification Reference 3.1.3

Exam Tip

Remember:
Saturated fatty acids have single bonds.
Unsaturated fatty acids have double bonds.

Triglyceride formation

Tip: There's more about condensation reactions on page 21.

Tip: You can pretty much ignore the 'R' group on the fatty acid — it never gets involved in the reaction.

Triglycerides are formed by condensation reactions. Figure 5 shows a fatty acid joining to a glycerol molecule. An **ester bond** forms between the two molecules, releasing a molecule of water — this is a condensation reaction. This process happens twice more to form a triglyceride.

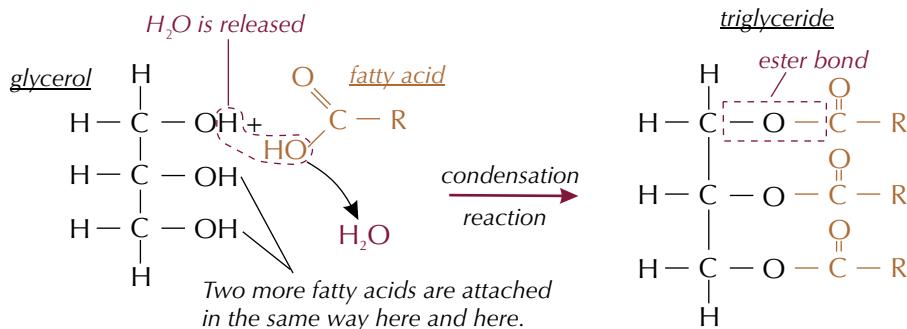


Figure 5: Triglyceride formation.

Phospholipids

The lipids found in cell membranes aren't triglycerides — they're phospholipids. Phospholipids are pretty similar to triglycerides except one of the fatty acid molecules is replaced by a phosphate group. The phosphate group is hydrophilic (attracts water). The fatty acid tails are hydrophobic (repel water). This is important in the cell membrane (see next page to find out why).

Tip: Remember, a phospholipid has a phosphate group.

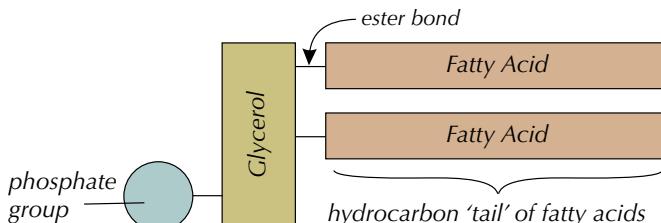


Figure 6: Structure of a phospholipid.

Properties of lipids

Tip: Lipids have many uses, including as certain hormones, such as testosterone, and as respiratory substrates (molecules used in respiration to release energy).

Tip: Storage molecules also need to be insoluble because otherwise they'd just dissolve (and release whatever they were storing) whenever they came into contact with water.

You need to know how the structures of triglycerides and phospholipids are related to their properties:

Triglycerides

Triglycerides are mainly used as energy storage molecules. They're good for this because the long hydrocarbon tails of the fatty acids contain lots of chemical energy — a load of energy is released when they're broken down. Because of these tails, lipids contain about twice as much energy per gram as carbohydrates.

Also, they're insoluble in water, so they don't affect the water potential (see p. 106) of the cell and cause water to enter the cells by osmosis (which would make them swell). The triglycerides bundle together as insoluble droplets in cells because the fatty acid tails are hydrophobic (water-repelling) — the tails face inwards, shielding themselves from water with their glycerol heads — see Figure 7.

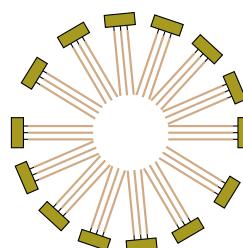


Figure 7: Diagram showing an insoluble triglyceride droplet.

Phospholipids

Phospholipids make up the bilayer of cell membranes (see p. 97).

Cell membranes control what enters and leaves a cell.

Phospholipid heads are hydrophilic and their tails are hydrophobic, so they form a double layer with their heads facing out towards the water on either side. The centre of the bilayer is hydrophobic, so water-soluble substances can't easily pass through it — the membrane acts as a barrier to those substances.

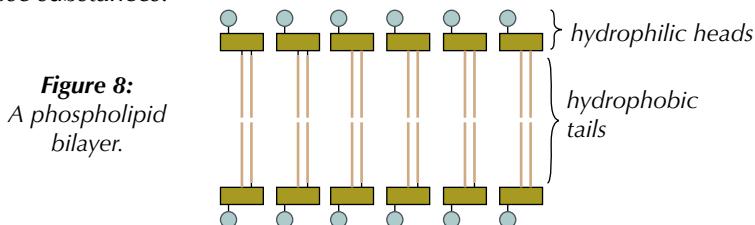


Figure 8:
A phospholipid bilayer.

Tip: There's more about the role of phospholipids in cell membranes on page 98.

The emulsion test for lipids

If you wanted to test for the presence of lipids in a sample, you'll need to do the **emulsion test**:

- Shake the test substance with ethanol for about a minute, then pour the solution into water.
- Any lipid will show up as a milky emulsion — see Figures 9 and 10.
- The more lipid there is, the more noticeable the milky colour will be.

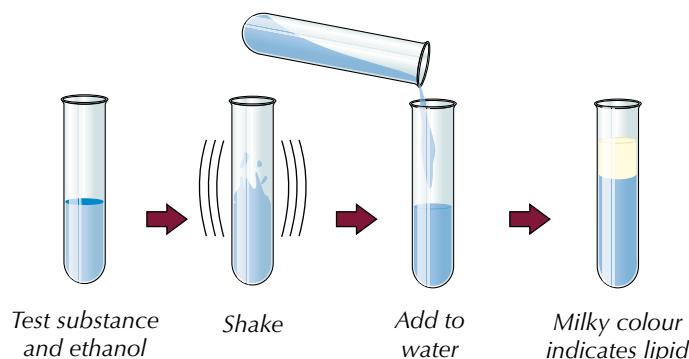


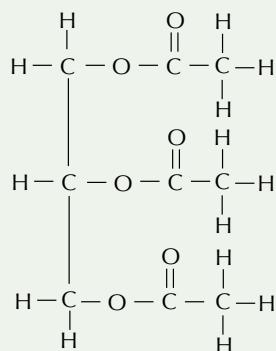
Figure 9: The emulsion test for lipids.



Figure 10: A positive result using the emulsion test.

Practice Questions — Application

- Q1 A triglyceride is shown on the right. Draw one molecule of the fatty acid that makes up the hydrocarbon tail.



Q2 The table below shows the structures of four fatty acids.

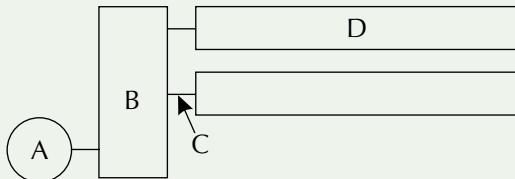
| Name: | Structure: |
|----------------|---|
| Propanoic acid | $\text{CH}_3\text{CH}_2\text{COOH}$ |
| Palmitic acid | $\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$ |
| Stearic acid | $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$ |
| Oleic acid | $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ |

Tip: For Q2 b), think about which part of the fatty acid molecule reacts with glycerol.

- Identify the 'R' groups in each of the fatty acids in the table.
- Draw the triglyceride that would be formed from condensation reactions between a molecule of glycerol and three molecules of propanoic acid.
- Unsaturated fatty acids will decolourise iodine solution. Which of the fatty acids in the table above will produce a positive result when added to iodine solution?

Practice Questions — Fact Recall

- Q1 What are the components of a triglyceride?
Q2 Explain the difference between a saturated fatty acid and an unsaturated fatty acid.
Q3 Name the structures A-D in the diagram of a phospholipid below.



- Q4 Give two reasons why triglycerides are used as energy storage molecules.
Q5 Explain how the structure of phospholipids makes them able to form the bilayer of cell membranes.
Q6 A student carries out an emulsion test on a food sample.
 - What is the student testing for?
 - Describe how the student should carry out the test and what he should expect to see if the result is positive.

5. Proteins

Proteins come in all shapes and sizes and have a huge variety of functions in all living organisms.

What are proteins made from?

The monomers of proteins are amino acids. A **dipeptide** is formed when two amino acids join together. A **polypeptide** is formed when more than two amino acids join together. Proteins are made up of one or more polypeptides.

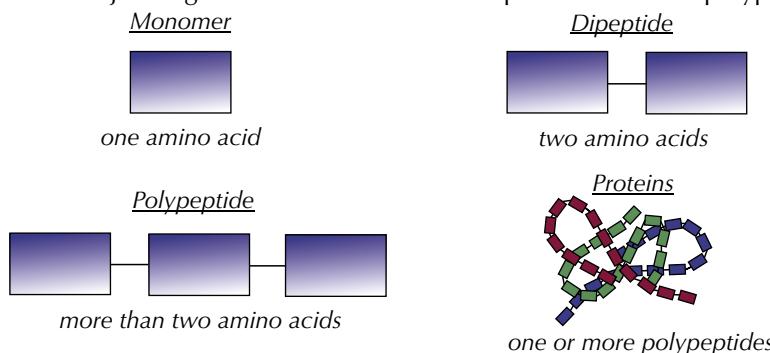


Figure 1: Amino acids join together to form peptides and proteins.

Amino acid structure

Amino acids have the same general structure — a carboxyl group (-COOH), an amine or amino group (-NH₂) and an R group (also known as a variable side group) attached to a carbon atom. R groups generally contain carbon. The only exception to this rule is glycine — its R group consists of just one hydrogen atom.

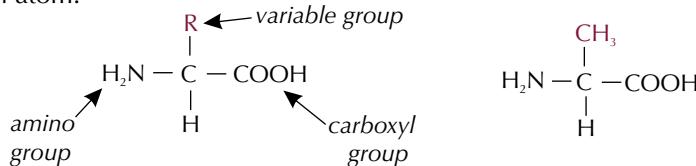


Figure 2: The general structure of an amino acid (left) and the structure of alanine (right).

All living things share a bank of only 20 amino acids. The only difference between them is what makes up their R group.

Dipeptide and polypeptide formation

Amino acids are linked together by **condensation reactions** (see page 21) to form dipeptides and polypeptides. A molecule of water is released during the reaction. The bonds formed between amino acids are called **peptide bonds**. The reverse reaction (hydrolysis) happens when dipeptides and polypeptides are broken down.

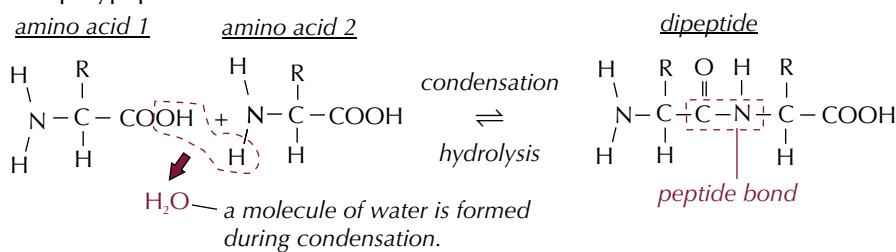


Figure 3: Dipeptide formation.

Learning Objectives:

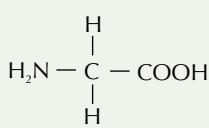
- Know that amino acids are the monomers from which proteins are made.
- Know that dipeptides are formed by the condensation of two amino acids, and polypeptides by the condensation of many amino acids.
- Know that a functional protein may contain one or more polypeptides.
- Know the general structure of an amino acid.
- Understand that the twenty amino acids that are common in all organisms differ only in their side group.
- Understand that a condensation reaction between two amino acids forms a peptide bond.
- Recall the role of hydrogen bonds, ionic bonds and disulfide bridges in the structure of proteins.
- Understand the relationship between primary, secondary, tertiary and quaternary protein structure, and protein function.
- Know that proteins have a variety of functions within all living organisms.
- Be able to relate the structure of proteins to the properties of proteins named throughout the specification.
- Recall the biuret test for proteins.

Specification Reference 3.1.4.1

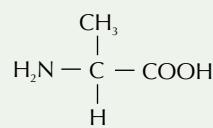
Practice Question — Application

Q1 Look at the following amino acid structures.

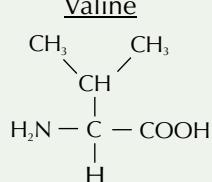
Glycine



Alanine



Valine



Draw the dipeptides and polypeptide that would be formed from a condensation reaction between:

- a) glycine and valine.
- b) alanine and glycine.
- c) glycine, alanine and valine.

Protein structure

Tip: Remember, proteins are polymers of amino acids (see page 21).

Tip: A hydrogen bond is a relatively weak bond formed between hydrogen atoms and other atoms, e.g. nitrogen or oxygen.

Tip: Think of the tertiary structure like a big, tangled-up spring.

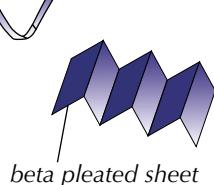
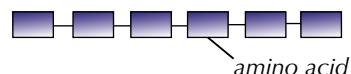
Tip: Disulfide bridges are covalent bonds between sulfur atoms. They're a lot stronger than the ionic and hydrogen bonds in proteins.

Tip: Not all proteins have a quaternary structure — some are made of only one polypeptide chain.

Proteins are big, complicated molecules. They're much easier to explain if you describe their structure in four 'levels'. These levels are a protein's primary, secondary, tertiary and quaternary structures.

Primary structure

This is the sequence of amino acids in the polypeptide chain.



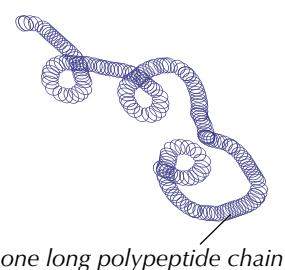
Secondary structure

The polypeptide chain doesn't remain flat and straight. Hydrogen bonds form between the amino acids in the chain. This makes it automatically coil into an alpha (α) helix or fold into a beta (β) pleated sheet — this is the secondary structure.

Tertiary structure

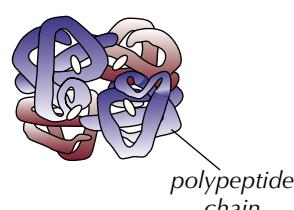
The coiled or folded chain of amino acids is often coiled and folded further. More bonds form between different parts of the polypeptide chain, including **hydrogen bonds** and **ionic bonds** (attractions between negative and positive charges on different parts of the molecule).

Disulfide bridges also form whenever two molecules of the amino acid cysteine come close together — the sulfur atom in one cysteine bonds to the sulfur atom in the other. For proteins made from a single polypeptide chain, the tertiary structure forms their final 3D structure.



Quaternary structure

Some proteins are made of several different polypeptide chains held together by bonds. The quaternary structure is the way these polypeptide chains are assembled together. For proteins made from more than one polypeptide chain (e.g. haemoglobin, insulin, collagen), the quaternary structure is the protein's final 3D structure.



Protein shape and function

A protein's shape determines its function — e.g. haemoglobin is a compact, soluble protein, which makes it easy to transport. This makes it great for carrying oxygen around the body (see p. 170). There are loads of different proteins found in living organisms. They've all got different structures and shapes, which makes them specialised to carry out particular jobs.

Exam Tip

You need to be able to relate the structure of a protein to its function for any protein you're given.

Examples

Enzymes — They're usually roughly spherical in shape due to the tight folding of the polypeptide chains. They're soluble and often have roles in metabolism, e.g. some enzymes break down large food molecules (digestive enzymes, see pages 166-168) and other enzymes help to synthesise (make) large molecules.

Antibodies — Antibodies are involved in the immune response and are found in the blood. They're made up of two light (short) polypeptide chains and two heavy (long) polypeptide chains bonded together. Antibodies have variable regions (see p. 119) — the amino acid sequences in these regions vary greatly.

Transport proteins — E.g. channel proteins are present in cell membranes (page 103). Channel proteins contain hydrophobic (water hating) and hydrophilic (water loving) amino acids, which cause the protein to fold up and form a channel (see Figure 5). These proteins transport molecules and ions across membranes.

Structural proteins — Structural proteins are physically strong. They consist of long polypeptide chains lying parallel to each other with cross-links between them. Structural proteins include keratin (found in hair and nails) and collagen (found in connective tissue). Collagen has three polypeptide chains tightly coiled together, which makes it strong. This makes it a great supportive tissue in animals.

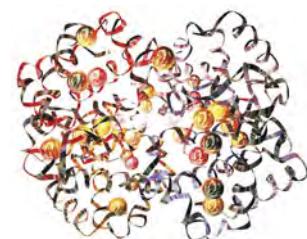


Figure 4: A molecular model of haemoglobin.

Tip: Proteins are also used as chemical messengers in the body, e.g. as hormones like insulin.

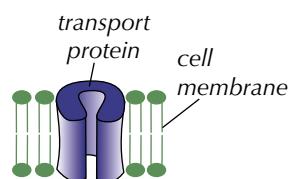


Figure 5: A transport protein in a cell membrane.

The biuret test for proteins

If you needed to find out if a substance, e.g. a food sample, contained protein you'd use the biuret test. There are two stages to this test.

1. The test solution needs to be alkaline, so first you add a few drops of sodium hydroxide solution.
2. Then you add some copper(II) sulfate solution.

If protein is present, the solution turns purple. If there's no protein, the solution will stay blue — see Figure 7. The colours can be fairly pale, so you might need to look carefully.

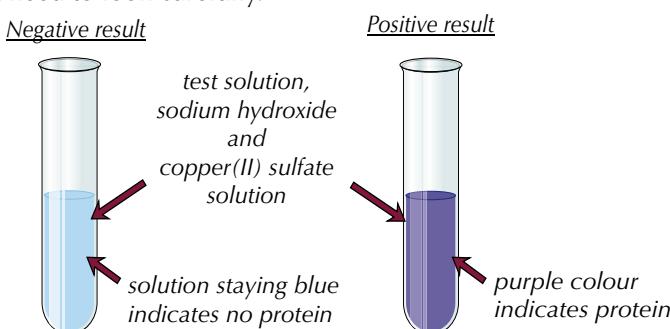


Figure 7: A negative and positive biuret test result.



Figure 6: A molecular model of collagen.

Tip: Remember to assess any hazards before doing tests like these. For example, if you're using dilute sodium hydroxide you'll need to wear safety goggles as it's an irritant.



Figure 8: A negative (left) and positive (right) biuret test result.

Exam Tip

When a question says 'suggest' you're not expected to know the exact answer — you're expected to use your knowledge to come up with a sensible answer.

Practice Questions — Application

A biuret test was carried out to determine which liquids contained protein. The results of the experiment are shown in the table below.

| Liquid | Result |
|------------------|--------|
| De-ionised water | Blue |
| Cow's milk | Blue |
| Orange juice | Purple |
| Orange squash | Blue |
| Goat's milk | Purple |

- Q1 Which of the liquids in the table gave a positive test result?
- Q2 Suggest why the scientist tested de-ionised water.
- Q3 The scientist measured the pH of each liquid after the test. The pH of the cow's milk was below 7, so the scientist marked the test result as void.
- Why did they mark the result as void?
 - Suggest what mistake the scientist might have made during the experiment.

Practice Questions — Fact Recall

- Q1 What are the monomers of proteins?
- Q2 What is a polypeptide?
- Q3 Draw the general structure of an amino acid.
- Q4 What sort of reaction links amino acids together?
- Q5 What is the name of the bond that forms between amino acids?
- Q6 Name three bonds that may be formed between the amino acids in a polypeptide chain to form the tertiary structure of a protein.
- Q7 Explain how the shape of structural proteins make them specialised for their function.
- Q8 The biuret test is used to test for proteins.
- What is added to the test solution to make it alkaline?
 - What is added next to the solution?
 - What would a positive test result look like?

Exam Tip

Make sure you know how to carry out the biuret test, as well as what a positive result and a negative result look like.

6. Enzymes

Enzymes are proteins that speed up the rate of chemical reactions.

Enzymes as biological catalysts

Enzymes speed up chemical reactions by acting as biological catalysts. They catalyse metabolic reactions — both at a cellular level (e.g. respiration) and for the organism as a whole (e.g. digestion in mammals). Enzymes can affect structures in an organism (e.g. enzymes are involved in the production of collagen, an important protein in the connective tissues of animals) as well as functions (like respiration). Enzyme action can be intracellular — within cells, or extracellular — outside cells.

Enzymes are proteins (see page 35). Enzymes have an **active site**, which has a specific shape. The active site is the part of the enzyme where the substrate molecules (the substance that the enzyme interacts with) bind to. Enzymes are highly specific due to their tertiary structure (see page 34).

How enzymes speed up reactions

In a chemical reaction, a certain amount of energy needs to be supplied to the chemicals before the reaction will start. This is called the **activation energy** — it's often provided as heat. Enzymes lower the amount of activation energy that's needed, often making reactions happen at a lower temperature than they could without an enzyme. This speeds up the rate of reaction.

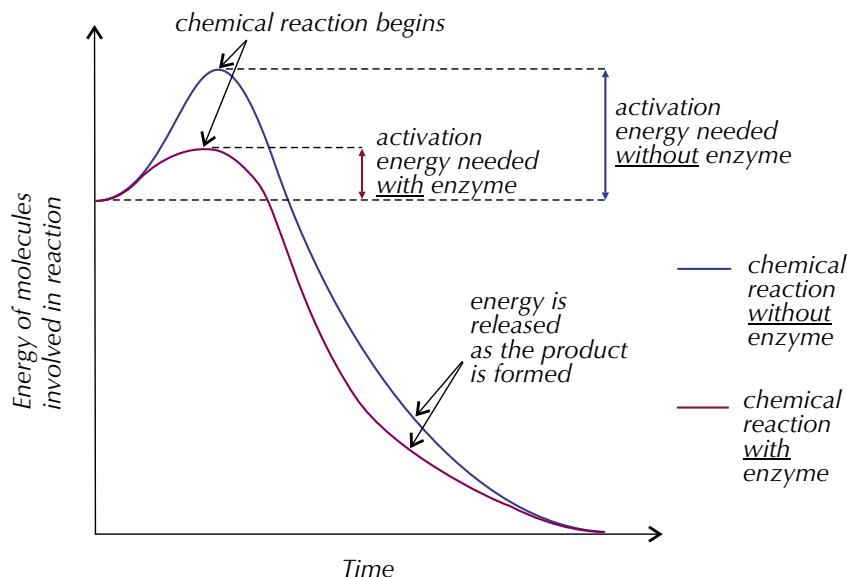


Figure 1: A graph to show the activation energy needed for a reaction with and without an enzyme.

When a substrate fits into the enzyme's active site it forms an **enzyme-substrate complex** — it's this that lowers the activation energy. Here are two reasons why:

- If two substrate molecules need to be joined, being attached to the enzyme holds them close together, reducing any repulsion between the molecules so they can bond more easily.
- If the enzyme is catalysing a breakdown reaction, fitting into the active site puts a strain on bonds in the substrate, so the substrate molecule breaks up more easily.

Learning Objectives:

- Be able to appreciate that enzymes catalyse a wide range of intracellular and extracellular reactions that determine structures and functions from cellular to whole-organism level.
- Know that each enzyme lowers the activation energy of the reaction it catalyses.
- Be able to appreciate how models of enzyme action have changed over time.
- Recall the induced-fit model of enzyme action.
- Understand the specificity of enzymes.
- Know how the properties of an enzyme relate to the tertiary structure of its active site and its ability to combine with complementary substrate(s) to form an enzyme-substrate complex.

Specification Reference 3.1.4.2

Tip: A catalyst is a substance that speeds up a chemical reaction without being used up in the reaction itself.

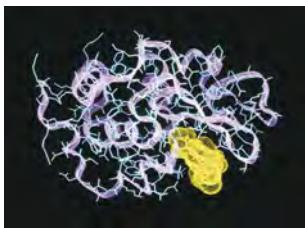


Figure 2: Computer model of an enzyme-substrate complex. The substrate (yellow) has bound to the enzyme's active site.

Exam Tip

When describing enzyme action you need to say the active site and the substrate have a complementary shape, rather than the same shape.

Tip: The diagrams on this page show how enzymes break substrates down (e.g. one substrate molecule goes into the active site and two products come out). Enzymes can also catalyse synthesis reactions (e.g. two substrate molecules go into the active site, bind together and one product comes out).

Tip: After the products are released, the active site returns to its original shape and can bind to the next substrate molecule.

Models of enzyme action

Scientists now have a pretty good understanding of how enzymes work. As with most scientific theories, this understanding has changed over time.

The 'lock and key' model

Enzymes are a bit picky — they only work with substrates that fit their active site. Early scientists studying the action of enzymes came up with the 'lock and key' model. This is where the substrate fits into the enzyme in the same way that a key fits into a lock — the active site and the substrate have a complementary shape.

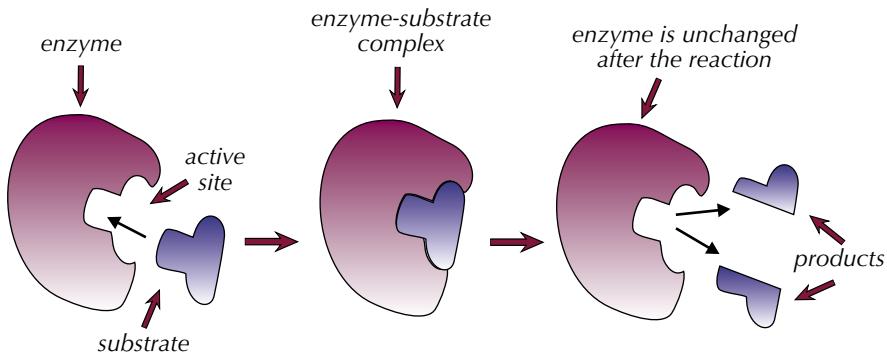


Figure 3: The 'lock and key' model.

Scientists soon realised that the lock and key model didn't give the full story. The enzyme and substrate do have to fit together in the first place, but new evidence showed that the enzyme-substrate complex changed shape slightly to complete the fit. This locks the substrate even more tightly to the enzyme. Scientists modified the old lock and key model and came up with the 'induced fit' model.

The 'induced fit' model

The 'induced fit' model helps to explain why enzymes are so specific and only bond to one particular substrate. The substrate doesn't only have to be the right shape to fit the active site, it has to make the active site change shape in the right way as well. This is a prime example of how a widely accepted theory can change when new evidence comes along. The 'induced fit' model is still widely accepted — for now, anyway.

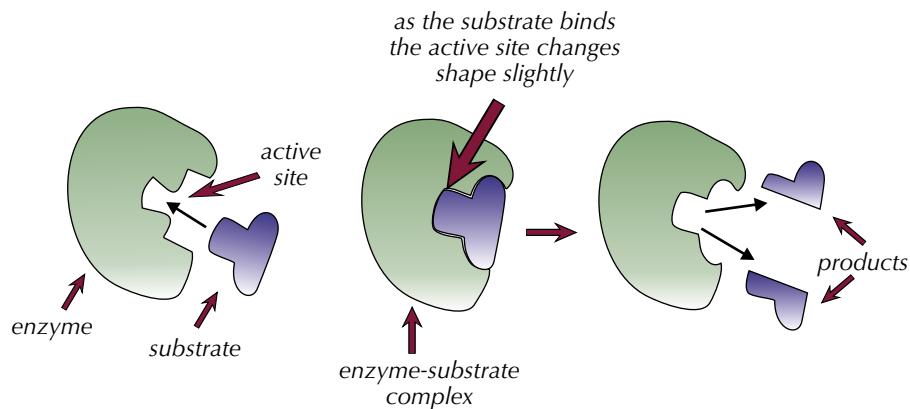


Figure 4: The 'induced fit' model.

Enzyme properties

Enzyme properties are related to their tertiary structure. Enzymes are very specific — they usually only catalyse one reaction, e.g. maltase only breaks down maltose, sucrase only breaks down sucrose. This is because only one complementary substrate will fit into the active site. The active site's shape is determined by the enzyme's tertiary structure (which is determined by the enzyme's primary structure). Each different enzyme has a different tertiary structure and so a different shaped active site. If the substrate shape doesn't match the active site, an enzyme-substrate complex won't be formed and the reaction won't be catalysed — see Figure 5.

Tip: See page 34 for more on the primary and tertiary structure of proteins.

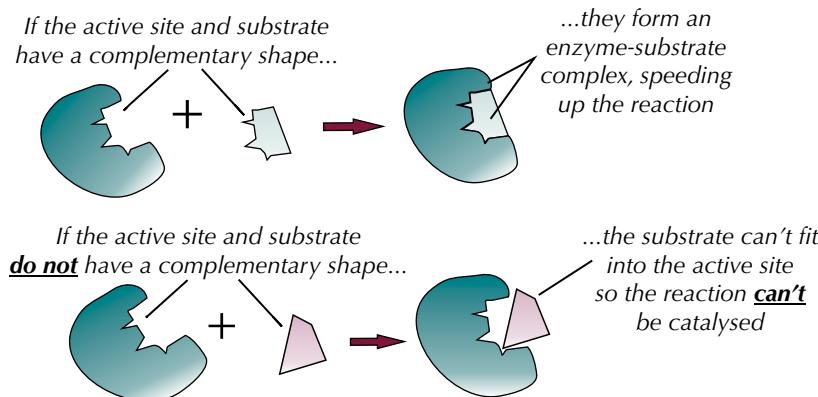


Figure 5: An enzyme's active site has a complementary shape to the substrate.

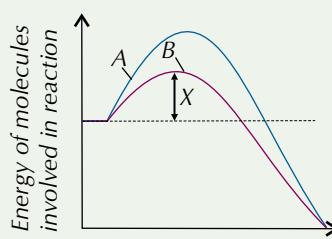
If the tertiary structure of a protein is altered in any way, the shape of the active site will change. This means the substrate won't fit into the active site, an enzyme-substrate complex won't be formed and the enzyme will no longer be able to carry out its function. The tertiary structure of an enzyme may be altered by changes in pH or temperature (see pages 40-41).

The primary structure (amino acid sequence) of a protein is determined by a gene. If a mutation occurs in that gene, it could change the tertiary structure of the enzyme produced.

Tip: See page 223 for more on mutations.

Practice Questions — Fact Recall

- Q1 What term is used to describe an enzyme that acts outside cells?
- Q2 Look at the graph on the right.
 - a) Which line shows a reaction with the presence of an enzyme?
 - b) What does the line labelled X represent?
- Q3 Explain, in terms of activation energy, why an enzyme enables reactions to happen at lower temperatures than they could without an enzyme.
- Q4 What is the main difference between the lock and key model and the induced fit model?
- Q5 What determines the shape of an enzyme's active site?
- Q6 Why will an enzyme only bind with one substrate?



Tip: If you're having problems getting your head around activation energy, just imagine you have to get to the top of a mountain to start a chemical reaction. It would take a lot of energy to get to the top. An enzyme effectively reduces the height of the mountain, so it doesn't take as much energy to start the reaction.

Learning Objective:

- Be able to describe and explain the effects of enzyme concentration, substrate concentration, concentration of competitive and of non-competitive inhibitors, pH and temperature on the rate of enzyme controlled reactions.

Specification Reference 3.1.4.2

Tip: In most cases, denaturation permanently changes an enzyme's shape, i.e. it won't go back to normal when the temperature decreases again.

Exam Tip

Make sure you don't say the enzyme's killed by high temperatures — it's denatured.

Exam Tip

You need to understand that different enzymes can have different optimum temperatures, but you don't have to learn any specific optimum temperature values.

7. Factors Affecting Enzyme Activity

Enzymes are great at speeding up reactions, but there are several factors that affect how fast they work.

Measuring enzyme activity

Measuring the rate of a reaction can be done in two ways:

1. How fast the product is made

There are different molecules present at the end of a chemical reaction than there are at the beginning. By measuring the amount of end product present at different times during the experiment the reaction rate can be calculated.

2. How fast the substrate is broken down

To produce the end products in a reaction, substrate molecules have to be used up. By measuring the amount of substrate molecules left at different times during the experiment the reaction rate can be calculated.

Temperature

Like any chemical reaction, the rate of an enzyme-controlled reaction increases when the temperature's increased. More heat means more kinetic energy, so molecules move faster. This makes the substrate molecules more likely to collide with the enzymes' active sites. The energy of these collisions also increases, which means each collision is more likely to result in a reaction.

But, if the temperature gets too high, the reaction stops. The rise in temperature makes the enzyme's molecules vibrate more. If the temperature goes above a certain level, this vibration breaks some of the bonds that hold the enzyme in shape. The active site changes shape and the enzyme and substrate no longer fit together. At this point, the enzyme is **denatured** — it no longer functions as a catalyst — see Figure 1.

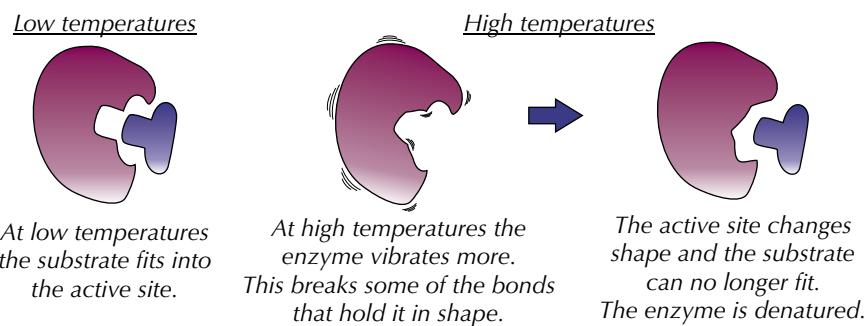


Figure 1: Effect of temperature on enzyme activity.

Every enzyme has an optimum temperature. For most human enzymes it's around 37 °C, but some enzymes, like those used in biological washing powders, can work well at 60 °C.

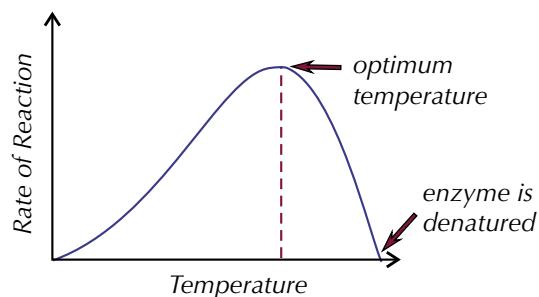


Figure 2: Effect of temperature on the rate of an enzyme-controlled reaction.

pH

All enzymes have an optimum pH value. Most human enzymes work best at pH 7 (neutral), but there are exceptions. Pepsin, for example, works best at pH 2 (acidic), which is useful because it's found in the stomach. Above and below the optimum pH, the H⁺ and OH⁻ ions found in acids and alkalis can disrupt the ionic bonds and hydrogen bonds that hold the enzyme's tertiary structure in place. The enzyme becomes denatured, and the active site changes shape.

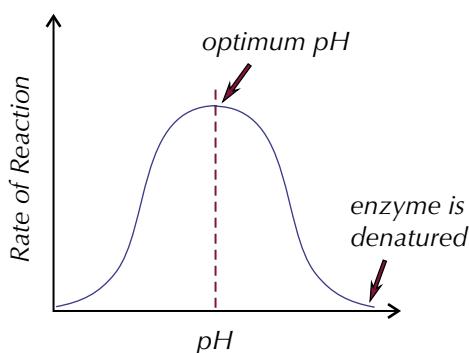


Figure 3: Effect of pH on the rate of an enzyme-controlled reaction.

Tip: Ionic bonds are attractions between negative and positive charges on different parts of the molecule (see p. 34).

Exam Tip

Don't forget — both a pH that's too high and one that's too low will denature an enzyme, not just one that's too high.

Substrate concentration

The higher the substrate concentration, the faster the reaction — more substrate molecules means a collision between substrate and enzyme is more likely and so more active sites will be occupied. This is only true up until a 'saturation' point though. After that, there are so many substrate molecules that the enzymes have about as much as they can cope with (all the active sites are full), and adding more makes no difference — see Figures 4 and 5.

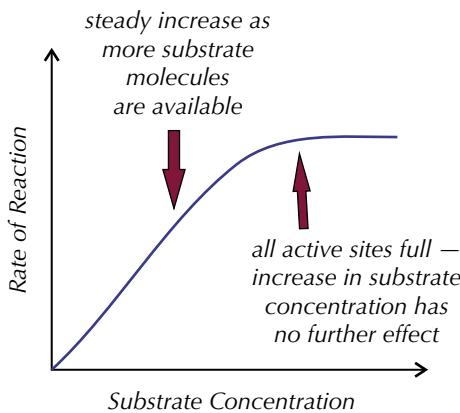
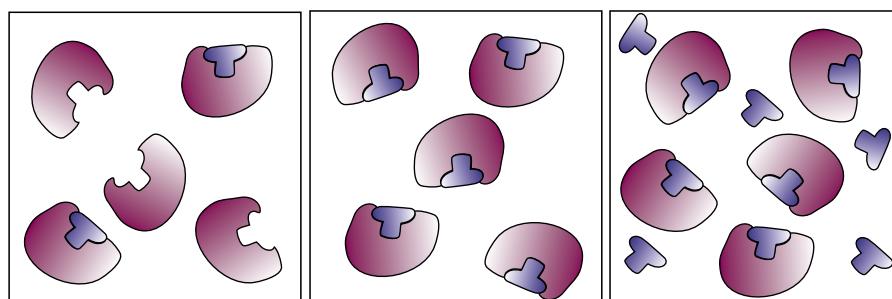


Figure 4: A graph to show the rate of an enzyme-controlled reaction against substrate concentration.

Tip: These graphs show the rate of reaction (i.e. the speed of the reaction). When the line on the graph plateaus it doesn't mean the reaction has stopped, just that it isn't going any faster.



Low substrate concentration — not all active sites are occupied.

Saturation point — all active sites are occupied.

Beyond saturation point — all active sites are occupied and there are spare substrate molecules.

Figure 5: Effect of substrate concentration on occupation of active sites.

Exam Tip

Don't ever say that the enzymes are used up — say that all the active sites are occupied.

Enzyme concentration

Tip: The enzyme concentration and substrate concentration graphs initially show a linear (straight line) relationship between the concentration and the rate of reaction. This means you can use the gradient of the line to work out how fast the rate is changing — see p. 12 for more.

The more enzyme molecules there are in a solution, the more likely a substrate molecule is to collide with one and form an enzyme-substrate complex. So increasing the concentration of the enzyme increases the rate of reaction.

But, if the amount of substrate is limited, there comes a point when there's more than enough enzyme molecules to deal with all the available substrate, so adding more enzyme has no further effect.

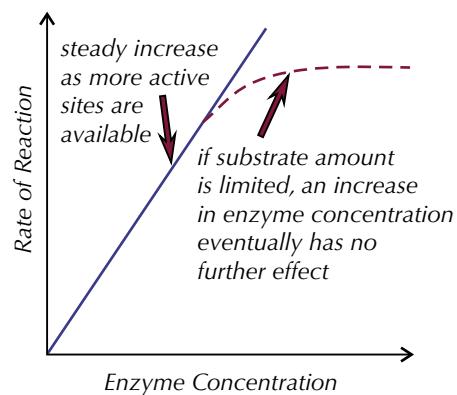


Figure 6: A graph to show the rate of an enzyme-controlled reaction against enzyme concentration.

Interpreting line graphs

You might be asked to interpret the graph of an enzyme-controlled reaction in the exam.

Example

You might be asked a question like this in the exam:

'Describe and explain the differences between the three curves shown on the graph below.'

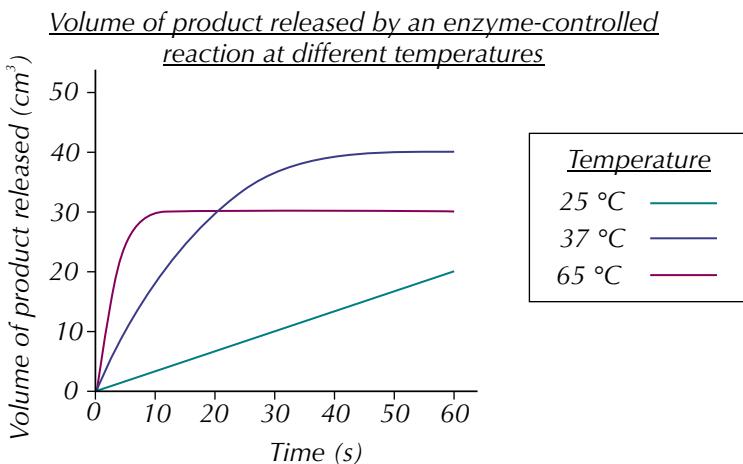
Tip: The graph on the right shows the release of a product over time.

Exam Tip

The graph in your exam could be based on any variable — e.g. pH, temperature, enzyme concentration or substrate concentration. You'll have to use your knowledge of enzymes to explain what's going on.

Exam Tip

You might have to work out the initial rate of reaction in the exam — see page 48 for how to do this.



Here's how to answer it:

1. Compare the rates of reaction at the start of the graph.

E.g. in the graph above, the rate of reaction at the start was fastest at 65 °C. This is because the molecules have more kinetic energy at 65 °C, so they are moving faster, meaning the substrate is more likely to collide with an enzyme's active site and more enzyme-substrate complexes are formed. More energy also means collisions are more likely to result in a reaction.

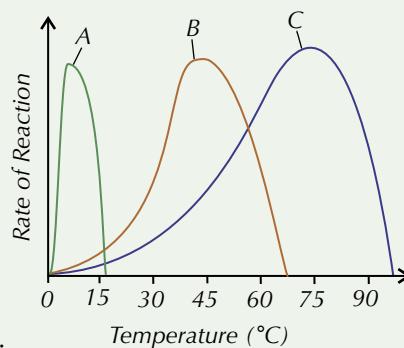
2. Look at the rest of the graph and compare the different temperatures.

At 37 °C the graph has plateaued (flattened out) after 40 s because all the substrate has been used up. At 65 °C the graph has plateaued earlier than at 37 °C (at about 10 s), because the high temperature caused the enzyme to denature, so the reaction stopped sooner. Not as much product was made because not all the substrate was converted to product before the enzyme was denatured, so there is still substrate left. At 25 °C the rate of reaction is remaining constant and the volume of product is continuing to increase because not all of the substrate has been used up.

Practice Questions — Application

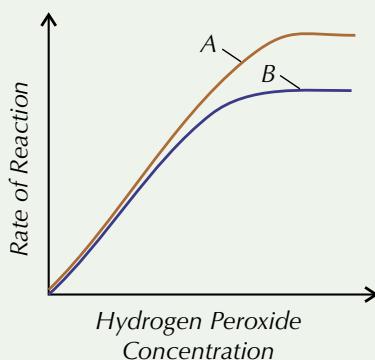
Q1 Hyperthermophilic bacteria are found in hot springs where temperatures reach 80 °C. Psychrotrophic bacteria are found in very cold environments. The graph on the right shows the rate of reaction for an enzyme from three different bacteria.

- Explain which curve on the graph shows the enzyme from:
 - hyperthermophilic bacteria.
 - psychrotrophic bacteria.
- Explain what would happen to enzyme activity for each type of bacteria shown on the graph if they were put into an environment with a temperature range of 60–75 °C.



Exam Tip

When you're asked to answer questions about a graph, use specific values in your answer where you can.



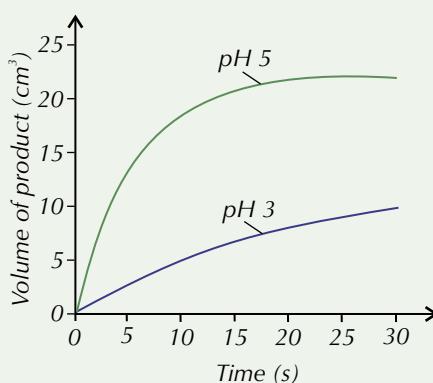
Q2 The graph on the left shows the rate of reaction for the enzyme catalase under two different conditions. Catalase is found in the liver.

- Explain which curve on the graph represents the reaction with the greatest concentration of catalase.
- Both of the curves flatten out. Explain why this is.

Tip: Catalase catalyses the breakdown of hydrogen peroxide.

Q3 A group of students were investigating the effect of pH on the efficiency of enzyme A. They measured the volume of product released from the enzyme-controlled reaction at two different pH values. Their results are shown in the graph on the right.

Describe and explain the differences between the curves.



Enzyme inhibitors

Enzyme activity can be prevented by enzyme inhibitors — molecules that bind to the enzyme that they inhibit. Inhibition can be competitive or non-competitive.

Exam Tip

Don't say that the inhibitor molecule and the substrate have the same shape — they have a similar shape.

Tip: If you have a competitive inhibitor, increasing the concentration of substrate will reverse its effects — the substrate will out-compete the inhibitor for the active site.

Competitive inhibitors

Competitive inhibitor molecules have a similar shape to that of substrate molecules. They compete with the substrate molecules to bind to the active site, but no reaction takes place. Instead they block the active site, so no substrate molecules can fit in it — see Figure 7.

How much the enzyme is inhibited depends on the relative concentrations of the inhibitor and substrate. If there's a high concentration of the inhibitor, it'll take up nearly all the active sites and hardly any of the substrate will get to the enzyme. But if there's a higher concentration of substrate, then the substrate's chances of getting to an active site before the inhibitor increase. So increasing the concentration of substrate will increase the rate of reaction (up to a point).

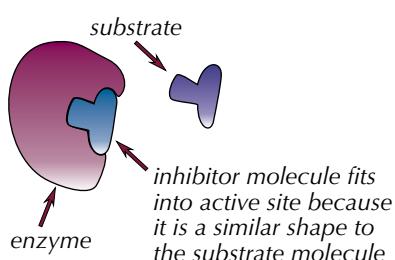


Figure 7: Competitive inhibition.

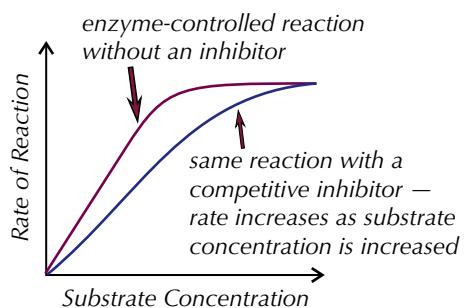


Figure 8: Effect of a competitive inhibitor on the rate of an enzyme-controlled reaction.

Exam Tip

When you're talking about shape change, always refer to the active site — don't just say the enzyme's changed shape.

Non-competitive inhibitors

Non-competitive inhibitor molecules bind to the enzyme away from its active site. This causes the active site to change shape so the substrate molecules can no longer bind to it — see Figure 9.

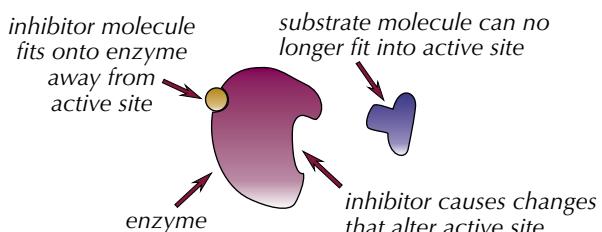


Figure 9: Non-competitive inhibition.

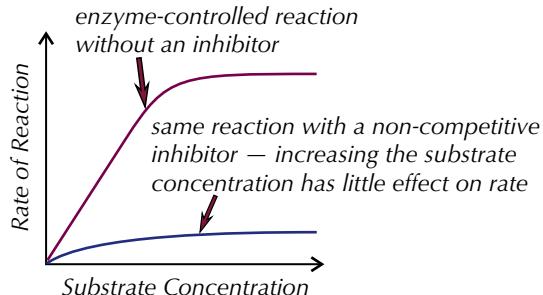


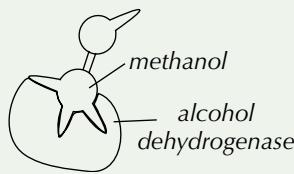
Figure 10: Effect of a non-competitive inhibitor on the rate of an enzyme-controlled reaction.

Non-competitive inhibitor molecules don't compete with the substrate molecules to bind to the active site because they are a different shape. Increasing the concentration of substrate won't make any difference — enzyme activity will still be inhibited.

Practice Questions — Application

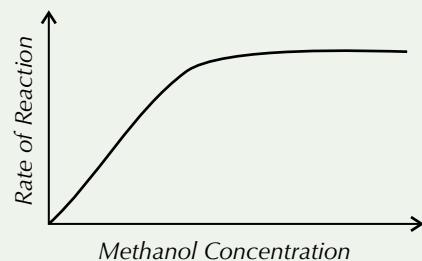
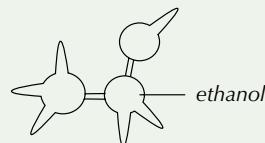
Methanol is broken down in the body into formaldehyde. The build up of formaldehyde can cause death. The enzyme that hydrolyses the reaction is alcohol dehydrogenase.

The enzyme-substrate complex formed is shown on the right.



Q1 A diagram of ethanol is shown on the right.

If someone had been poisoned with methanol, they could be helped by being given ethanol as soon as possible. Explain why.



Q2 The graph shows the rate of the reaction with no ethanol present. Sketch a graph with the same axes showing the rate of reaction with the presence of ethanol.

Exam Tip

This is exactly the kind of question you could get in the exam — enzyme-inhibition is a favourite with examiners, so make sure you know it inside out.

Practice Questions — Fact Recall

- Q1 Explain why an increase in temperature increases the rate of enzyme activity.
- Q2 Explain how a very high temperature can stop an enzyme from working.
- Q3 What happens to an enzyme's shape and function when it is denatured?
- Q4 Give a factor other than temperature that can denature an enzyme.
- Q5 What is meant by the 'saturation point' in an enzyme controlled reaction?
- Q6 Explain what happens to the rate of an enzyme-controlled reaction when the substrate concentration is increased after the saturation point.
- Q7 Explain the effect of increasing the enzyme concentration on the rate of an enzyme-controlled reaction.
- Q8 Where do the following molecules bind to an enzyme:
 - a) a non-competitive inhibitor?
 - b) a competitive inhibitor?
- Q9 Explain how non-competitive inhibition prevents enzyme activity.

Exam Tip

Don't get confused when talking about the active site — it's always on the enzyme, not on the substrate.

Tip: There are lots of similar sounding words here — look back through the section if you're struggling to remember the difference between them.

Learning Objective:

- Be able to investigate the effect of a named variable on the rate of an enzyme-controlled reaction (Required Practical 1).

Specification Reference 3.1.4.2

Tip: If you're measuring the rate of a reaction, you need to find out how much the amount of reactant or product is changing over time.

Tip: Don't forget to do a risk assessment before you do either this experiment or the one on the next page. You should always take basic safety precautions like wearing goggles and a lab coat.

Tip: Enzymes can irritate the skin and may cause an allergic reaction, so they need to be handled with care.

Tip: A buffer solution is able to resist changes in pH when small amounts of acid or alkali are added.

Tip: A negative control reaction, i.e. a boiling tube containing hydrogen peroxide but no catalase, should also be carried out at each temperature.

8. Enzyme-Controlled Reactions

You need to know how to measure the effect of any of the variables described on pages 40-42 on the rate of an enzyme-controlled reaction.

Measuring the rate of an enzyme-controlled reaction

REQUIRED PRACTICAL 1

You know from page 40 that there are two ways of measuring the rate of an enzyme-controlled reaction, so here are some more details about both of those ways:

1. You can measure how fast the product of the reaction appears and use this to compare the rate of reaction under different conditions.

Example

Catalase catalyses the breakdown of hydrogen peroxide into water and oxygen. It's easy to measure the volume of oxygen produced and to work out how fast it's given off. In this experiment, you'll be working out the rate of reaction at different temperatures. Figure 1 shows the apparatus you'll need. The oxygen released displaces the water from the measuring cylinder. (A stand and clamp would also be pretty useful to hold the cylinder upside down, as would a stopwatch and a water bath.)

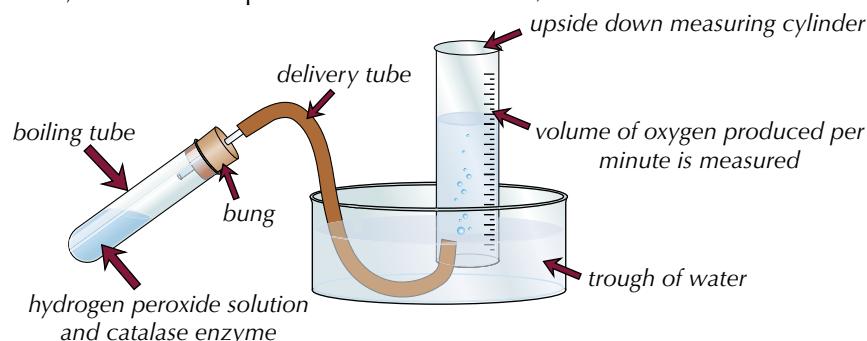


Figure 1: Apparatus needed for investigating the breakdown of hydrogen peroxide.

Here's how to carry out the experiment:

1. Set up boiling tubes containing the same volume and concentration of hydrogen peroxide. To keep the pH constant, add equal volumes of a suitable buffer solution to each boiling tube.
2. Set up the rest of the apparatus as shown in the diagram.
3. Put each boiling tube in a water bath set to a different temperature (e.g. 10 °C, 20 °C, 30 °C and 40 °C) along with another tube containing catalase. Wait 5 minutes before moving onto the next step so the enzyme gets up to temperature.
4. Use a pipette to add the same volume and concentration of catalase to each boiling tube. Then quickly attach the bung and delivery tube.
5. Record how much oxygen is produced in the first minute (60 s) of the reaction. Use a stopwatch to measure the time.
6. Repeat the experiment at each temperature three times, and use the results to find the mean volume of oxygen produced.
7. Calculate the mean rate of reaction at each temperature by dividing the volume of oxygen produced by the time taken (i.e. 60 s). The units will be $\text{cm}^3 \text{s}^{-1}$.

- You can measure how fast the substrate is broken down and use this to compare the rate of reaction under different conditions.

Example

The enzyme amylase catalyses the breakdown of starch to maltose. You can test for the presence of starch in a solution using iodine in potassium iodide solution — in this experiment, you're using this to work out the rate of reaction at different concentrations of enzyme. Figure 2 shows how the experiment can be set up. You'll need the apparatus shown in Figure 2 as well as a stopwatch.

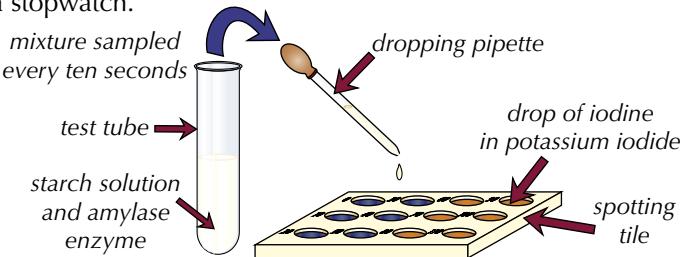


Figure 2: Apparatus needed for investigating the breakdown of starch.

- Put a drop of iodine in potassium iodide solution into each well on a spotting tile. Label the wells to help you read your results.
- Mix together a known concentration and volume of amylase and starch in a test tube.
- Use a dropping pipette to put a drop of this mixture into one of the wells containing the iodine solution at regular intervals (e.g. every 10 seconds).
- Observe the resulting colour. The iodine solution goes dark blue-black when starch is present but remains its normal browny-orange colour when there's no starch around.
- You can see how fast amylase is working by recording how long it takes for the iodine solution to no longer turn blue-black when the starch/amylase mixture is added.

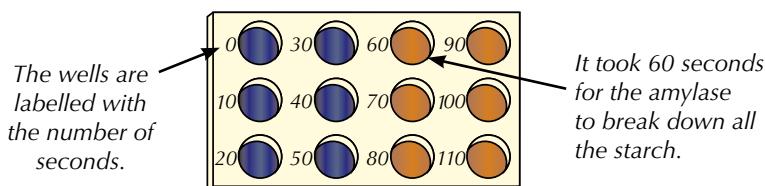


Figure 3: Example results from investigating the breakdown of starch.

- Repeat the experiment using different concentrations of amylase.
- Make sure that you also repeat the experiment three times at each amylase concentration and use your results to find the mean time taken.

Variables

The experiments above and on the previous page show you how you can investigate the effects of temperature and enzyme concentration on the rate of enzyme-controlled reactions.

You can also alter these experiments to investigate the effect of a different variable, such as pH (by adding a buffer solution with a different pH to each test tube or boiling tube) or substrate concentration (you could use serial dilutions to make substrate solutions with different concentrations). The key to experiments like this is to remember to only change one variable — everything else should stay the same.

Tip: Which method you use to measure the rate of a reaction will normally depend on whether the product or the substrate is easier to test for.

Exam Tip

You might have learnt different methods for measuring the rate of an enzyme-controlled reaction to those shown here and on the previous page — it doesn't matter which ones you revise, so long as you know them well enough to describe in the exam.

Tip: This experiment uses the starch test — see page 28.

Tip: There's more about controlling variables on page 1.

Tip: You can read all about how to make serial dilutions on page 107.

Estimating the initial rate of reaction

Tip: For more details on how to draw a tangent, see pages 13-14.

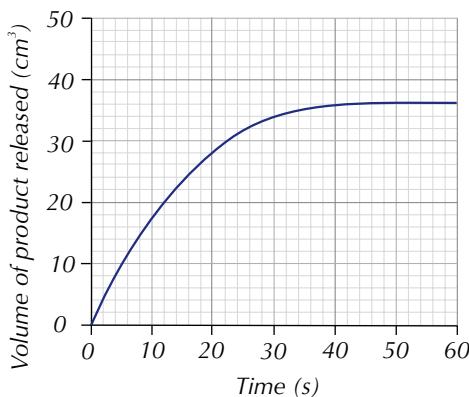
Tip: When you're working out a rate, the variable on the x axis should always be time.

Tip: If you're comparing the initial rate of reaction for two different reactions, you can work out the ratio of the rates to give you a quick and easy comparison.

E.g. if the initial rate of a reaction at 30 °C is $1.2 \text{ cm}^3 \text{ s}^{-1}$ and the initial rate at 60 °C is $3.0 \text{ cm}^3 \text{ s}^{-1}$, you could write the ratio of the initial rates of reaction at 30 °C : 60 °C as 1 : 2.5. There's more about working out ratios on page 7.

Example — Maths Skills

The graph below shows the volume of product released by an enzyme-controlled reaction at 37 °C.



To work out the initial rate of reaction:

1. Draw a tangent at $t = 0$.

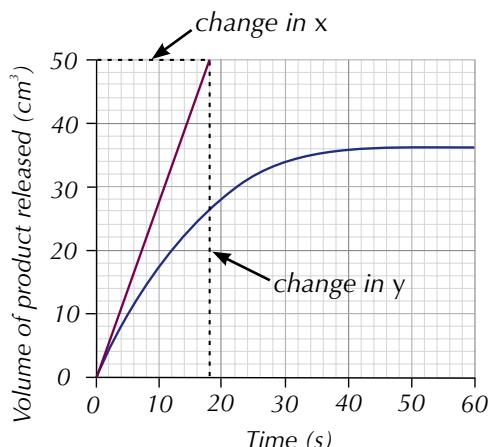
(See the red line on the graph on the right.)

2. Calculate the gradient of the tangent.

The gradient at $t = 0$ is:
 $\text{change in } y \div \text{change in } x$
 $= 50 \div 18 = 2.8$

3. Work out the units.

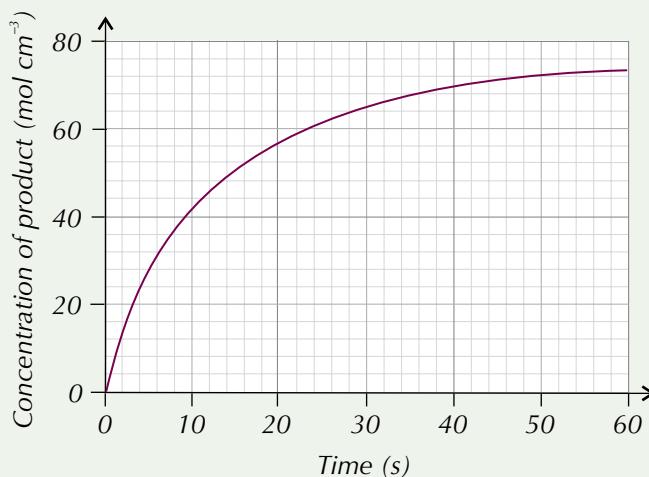
units of $y \div$ units of x
 $= \text{cm}^3 \div \text{s} = \text{cm}^3 \text{ s}^{-1}$



So the initial rate of reaction is $2.8 \text{ cm}^3 \text{ s}^{-1}$.

Practice Questions — Application

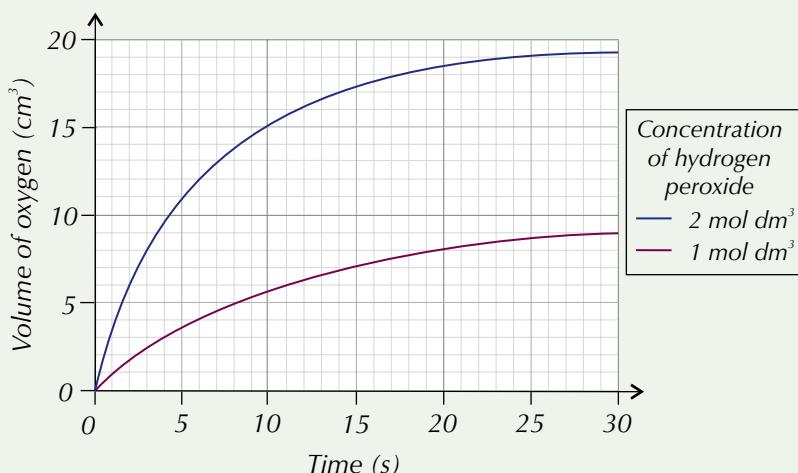
- Q1 The graph below shows the increase in the concentration of product from an enzyme-catalysed reaction at 25 °C.



Tip: Use a pencil to draw your tangents — then if you make a mistake or aren't happy with your line, you can just erase it and start again.

Use this graph to calculate the initial rate of reaction.

- Q2 A group of students were investigating the effect of hydrogen peroxide concentration on the rate of breakdown of hydrogen peroxide by the enzyme catalase. They measured the volume of oxygen released by the reaction. Their results are shown in the graph below.



Exam Tip

Don't forget to add the correct units to your answer, or you may miss out on some easy marks.

- Name two variables that the students should keep the same during this investigation.
- Calculate the ratio of the initial rates of reaction at 2 mol dm⁻³ : 1 mol dm⁻³ hydrogen peroxide.
Write your answer in the form X : 1.

Practice Question — Fact Recall

- Q1 Describe how you could measure the rate of the breakdown of hydrogen peroxide by catalase at different temperatures, including the equipment you would use.

Section Summary

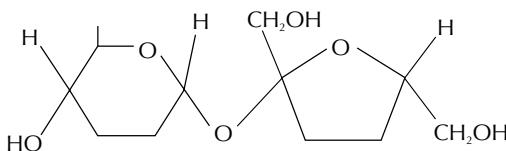
Make sure you know...

- That the biochemical basis of life is similar for all living things, providing evidence for evolution.
- That polymers are big molecules made from large numbers of smaller units called monomers.
- That monosaccharides, amino acids and nucleotides are examples of monomers.
- What is meant by a condensation reaction and a hydrolysis reaction, and how they work.
- That the monomers of carbohydrates are monosaccharides, e.g. glucose, galactose and fructose.
- The structure of α -glucose and the structure of β -glucose.
- That disaccharides are formed from the condensation reaction of two monosaccharides (forming glycosidic bonds) and which monosaccharides make up maltose, sucrose and lactose.
- How the Benedict's test for sugars is carried out and how to interpret the results.
- That polysaccharides are formed from the condensation of more than two monosaccharides.
- That starch and glycogen are formed by the condensation of α -glucose units.
- That cellulose is formed by the condensation of β -glucose units.
- How the structures of glycogen, starch and cellulose relate to their functions in animal and plant cells.
- How the iodine test for starch is carried out and how to interpret the results (blue-black = positive).
- That triglycerides and phospholipids are two groups of lipid.
- The basic structure of triglycerides (glycerol and three fatty acids) and how they're formed.
- The basic structure of fatty acids, including if they're saturated or unsaturated, and be able to recognise them.
- That a condensation reaction between glycerol and a fatty acid forms an ester bond.
- The basic structure of a phospholipid (glycerol, two fatty acids and a phosphate group).
- The different properties of triglycerides and phospholipids, and how they relate to their structures.
- How to carry out an emulsion test — shake sample with ethanol, add to water, milky = lipid present.
- That the monomers of proteins are amino acids, and how dipeptides and polypeptides are formed.
- That a functional protein may contain one or more polypeptides.
- The general structure of an amino acid and that they differ only in their side (R) group.
- How condensation reactions link amino acids together with peptide bonds.
- The relationship between primary, secondary, tertiary and quaternary protein structure.
- That the tertiary structure of proteins is held by hydrogen bonds, ionic bonds and disulfide bridges.
- That proteins have a variety of functions within all living organisms, and how their functions are related to their shape.
- How to carry out a biuret test for proteins and how to interpret the results (blue = negative result and purple = positive result).
- That enzymes catalyse a wide range of intracellular and extracellular reactions that determine structures and functions from cellular to whole-organism level.
- How enzymes catalyse reactions by lowering the activation energy of reactions.
- What the induced fit model of enzyme action is and how models of enzyme action have changed over time (from the lock and key model).
- Why enzymes are very specific and how the properties of enzymes relate to their tertiary structure (and their ability to form enzyme-substrate complexes).
- How temperature, pH, substrate concentration, enzyme concentration, and competitive and non-competitive inhibitors affect enzyme activity, and how to investigate these variables (Required Practical 1).
- How to draw a tangent to a graph and use it to work out the initial rate of a reaction.

Exam-style Questions

- 1 Disaccharides and polysaccharides are made from monosaccharides. **Figure 1** shows the disaccharide molecule sucrose.

Figure 1



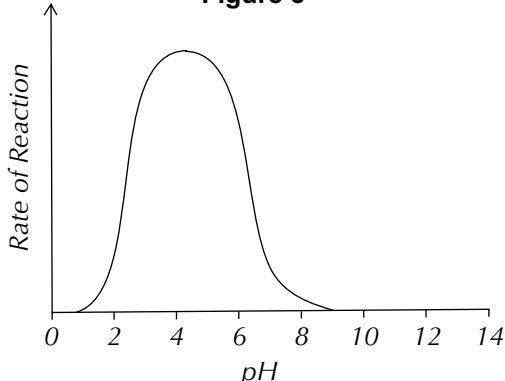
- 1.1 Describe the structure of sucrose and explain how it's formed. (4 marks)
- 1.2 Draw the **two** monosaccharides that join together to form sucrose. (2 marks)
- 1.3 Sucrose is a non-reducing sugar.
Describe a biochemical test you could use to identify the presence of a non-reducing sugar. (5 marks)
- 1.4 Glycogen is a polysaccharide.
Describe the structure of glycogen and explain how its structure makes it suited to its function. (4 marks)
- 2 Proteins are important biological molecules.
- 2.1 The biuret test can be used to test for the presence of protein in a sample.
Describe how this test would be carried out, including what observations would indicate positive and negative results. (4 marks)
- Enzymes are proteins. Pepsin is an enzyme released in the stomach to break down other proteins into smaller polypeptides. **Figure 2** shows a simplified diagram of the action of pepsin.
- Figure 2**
-
- The diagram illustrates the proteolytic action of pepsin. It shows a protein chain composed of three circles labeled 'A' being cleaved by a pepsin molecule (represented by a wavy shape). In the first step, the pepsin is shown approaching the protein. In the second step, the protein is cleaved into two shorter fragments, each consisting of two circles. Arrows indicate the cleavage sites.
- 2.2 Name the type of monomer represented by the letter A in **Figure 2** and draw its general structure. (2 marks)
- 2.3 Describe the process by which pepsin breaks down a protein. (3 marks)
- 2.4 Describe an enzyme's tertiary structure and how it relates to its properties. (5 marks)

- 3 Triglycerides are a type of fat found in foods. In the stomach, gastric lipase acts as a catalyst to break triglycerides down into diglycerides and fatty acids.



Figure 3 shows the rate of reaction for gastric lipase at different pH values.

Figure 3



- 3.1 What is the optimum pH of gastric lipase?

(1 mark)

- 3.2 At what pH value(s) is gastric lipase denatured? Give a reason for your answer.

(2 marks)

- 3.3 Explain what happens when an enzyme is denatured.

(2 marks)

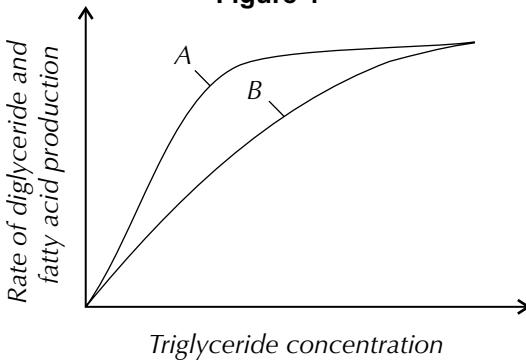
- 3.4 Suggest **two** variables you would control if you were investigating the activity of gastric lipase at different pH values.

(2 marks)

The weight-loss drug, orlistat, stops triglycerides from being broken down. Orlistat is a competitive inhibitor of gastric lipase.

Figure 4 shows the reaction with and without orlistat present.

Figure 4



- 3.5 Which curve on the graph shows the reaction without the presence of orlistat? Give a reason for your answer.

(1 mark)

- 3.6 Explain the action of orlistat in this reaction.

(3 marks)

Topic 1 B: More Biological Molecules

1. DNA and RNA

DNA and RNA are both essential for the function of living organisms...

DNA and RNA function

DNA and RNA are both types of nucleic acid. They're found in all living cells and they both carry information.

Your **DNA (deoxyribonucleic acid)** is used to store your genetic information — that's all the instructions needed to grow and develop from a fertilised egg to a fully grown adult. There's more on the role of DNA on p. 204.

RNA (ribonucleic acid) is similar in structure to DNA. One of its main functions is to transfer genetic information from the DNA to the ribosomes. Ribosomes are the body's 'protein factories' — they read the RNA to make polypeptides (proteins) in a process called translation (see pages 209-210). Ribosomes themselves are made from RNA and proteins.

Nucleotide structure

Molecules of DNA and RNA are polymers of nucleotides. A nucleotide is a type of biological molecule which is made from three different components: a pentose sugar (that's a sugar with 5 carbon atoms), a nitrogen-containing organic base (organic means that it contains carbon), and a phosphate group — see Figure 1.

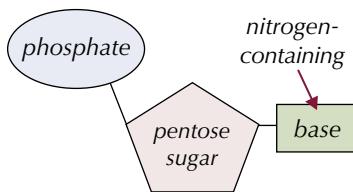


Figure 1: A nucleotide.

Nucleotides are really important. For a start they're the monomers (see p. 21) that make up DNA and RNA.

Polynucleotide structure

Many nucleotides join together to form polynucleotide strands (or chains). The nucleotides join up via a condensation reaction (see p. 21) between the phosphate group of one nucleotide and the sugar of another. This forms a phosphodiester bond (consisting of the phosphate group and two ester bonds). The chain of phosphates and sugars is known as the **sugar-phosphate backbone** — see Figure 2.

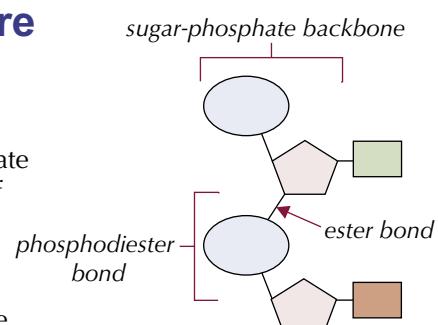


Figure 2: Structure of a single polynucleotide strand.

Learning Objectives:

- Understand that deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are important information-carrying molecules. In all living cells, DNA holds genetic information and RNA transfers genetic information from DNA to the ribosomes.
- Know that ribosomes are formed from RNA and proteins.
- Know that both DNA and RNA are polymers of nucleotides.
- Know the components of a nucleotide, and how they differ in DNA and RNA nucleotides.
- Know that a condensation reaction between two nucleotides forms a phosphodiester bond.
- Recall that a DNA molecule is a double helix with two polynucleotide chains held together by hydrogen bonds between specific complementary base pairs.
- Know that an RNA molecule is a relatively short polynucleotide chain.
- Appreciate that the relative simplicity of DNA led many scientists to doubt that it carried the genetic code.

Specification Reference 3.1.5.1

DNA structure

DNA has a **double helix** structure.

This means that a DNA molecule is formed from two separate strands which wind around each other to form a spiral (see Figure 3).

The strands are polynucleotides. They're made up of lots of nucleotides joined together in a long chain — see previous page.

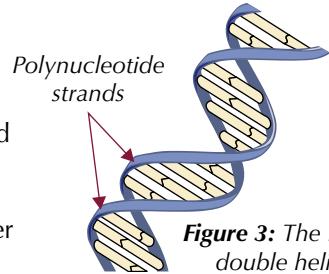


Figure 3: The DNA double helix.

DNA molecules are really long and are coiled up very tightly, so a lot of genetic information can fit into a small space in the cell nucleus.

DNA nucleotide structure

A DNA nucleotide is made from a phosphate group, the pentose sugar **deoxyribose** and a nitrogen-containing organic **base**.

Each DNA nucleotide has the same sugar and phosphate. The base on each nucleotide can vary though. There are four possible bases — adenine (A), thymine (T), cytosine (C) and guanine (G). The structure of a DNA nucleotide is illustrated in Figure 4.

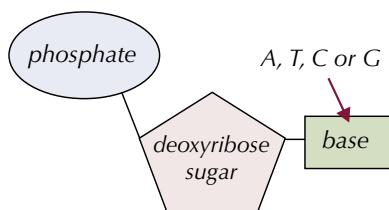


Figure 4: A DNA nucleotide.

Complementary base pairing

Tip: If you're struggling to remember which base pairs with which, just think — you eat Apple Turnover with Gloopy Custard.

Tip: The two ends of a polynucleotide strand are different — one end has a phosphate group and the other has a hydroxyl (OH) group attached to the sugar. That's how you can tell which direction a strand is running in.

Two DNA polynucleotide strands join together by hydrogen bonds between the bases. Each base can only join with one particular partner — this is called complementary base pairing (or specific base pairing). Adenine always pairs with thymine (A - T) and guanine always pairs with cytosine (G - C) — see Figure 5. This means there are always equal amounts of adenine and thymine in a DNA molecule and equal amounts of cytosine and guanine. Two hydrogen bonds form between A and T, and three hydrogen bonds form between C and G.

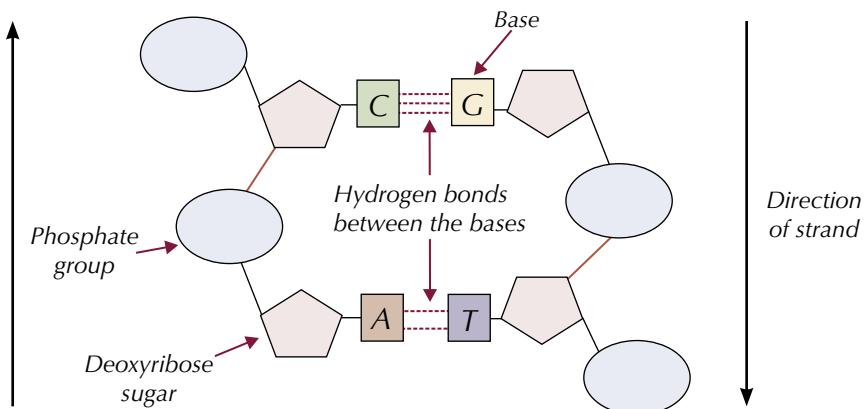


Figure 5: Complementary base pairing in DNA molecules.

The two polynucleotide strands are antiparallel — they run in opposite directions. Two antiparallel strands twist to form a DNA double helix.

Summary of a DNA molecule

If you tie all this information together, you end up with a DNA molecule that looks like the one in Figure 6.

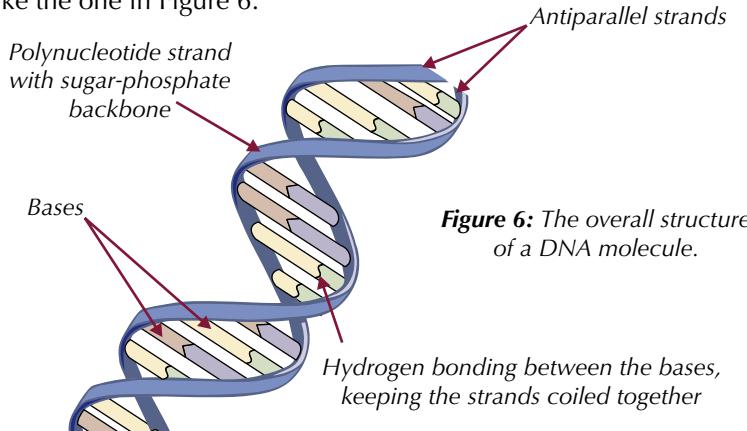


Figure 6: The overall structure of a DNA molecule.

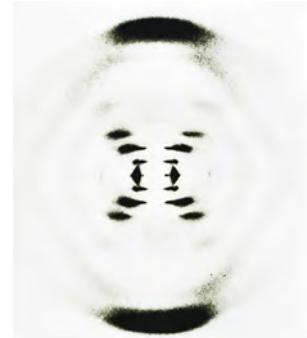


Figure 7: X-ray diffraction picture of DNA taken by Rosalind Franklin. The cross of bands shows that the molecule is a helix.

Practice Questions — Application

- Q1 Here are the base sequences of two short stretches of DNA. For each one, write down the sequence of bases they would pair up with:
a) ACTGTCGTAGTCGATGCTA b) TGCACCATGTGGTAAATCG
- Q2 Scientists analysed a section of double stranded DNA. There were 68 bases in total (34 base pairs) and 22 of the bases were adenine. How many of the bases were:
a) thymine? b) cytosine? c) guanine?

Tip: The structure of a nucleotide and the arrangement of the DNA double helix is the same in all living organisms.

RNA structure

Like DNA, RNA is made of nucleotides that contain a sugar, a phosphate group and one of four different bases. The nucleotides also form a polynucleotide strand with a sugar-phosphate backbone. But the structure of RNA differs from DNA in four main ways:

- The sugar in RNA nucleotides is a **ribose** sugar (not deoxyribose). It's still a pentose sugar though.
- **Uracil** (U, a pyrimidine) replaces thymine as a base. Uracil always pairs with adenine in RNA.
- The nucleotides form a single polynucleotide strand (not a double one).
- RNA strands are much **shorter** than most DNA polynucleotides.

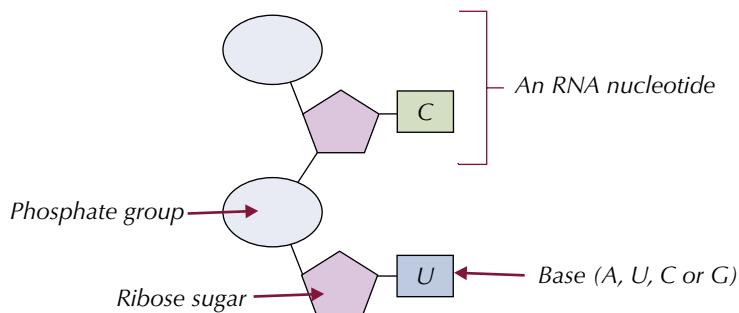


Figure 8: Structure of an RNA strand.

DNA and RNA comparison

Questions asking you to compare the structure of DNA and RNA are a regular feature in the exam. Luckily for you, the main points are summarised in the table below:

| | DNA | RNA |
|---------------|---|------------------|
| Shape | Double-stranded — twisted into a double helix and held together by hydrogen bonds | Single-stranded |
| Pentose sugar | Deoxyribose sugar | Ribose sugar |
| Bases | A, T, C, G | A, U, C, G |
| Size | Long | Relatively short |



Figure 9: Watson and Crick, two of the scientists who discovered the structure of DNA, and their model of the DNA double helix.

Exam Tip

Questions on the structure of DNA and RNA are easy marks in the exam — and they come up a lot. Make sure you know the structures inside out.

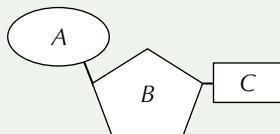
DNA as the carrier of the genetic code

DNA was first observed in the 1800s, but lots of scientists at the time doubted that it could carry the genetic code because it has a relatively simple chemical composition. Some argued that genetic information must be carried by proteins — which are much more chemically varied.

By 1953, experiments had shown that DNA was the carrier of the genetic code. This was also the year in which the double helix structure, which helps DNA to carry out its function, was determined by scientists James Watson and Francis Crick (see Figure 9).

Practice Questions — Fact Recall

- Q1 What is the function of DNA?
- Q2 What are ribosomes made up of?
- Q3 Name the monomer of DNA and RNA.
- Q4 The diagram below shows the structure of a nucleotide.



- Name parts A, B and C.
- Q5 Describe the structure of a DNA nucleotide.
 - Q6 Name the four possible bases in DNA.
 - Q7 What type of bond, present in a polynucleotide chain, consists of two ester bonds and a phosphate group?
 - Q8 Describe how a DNA double helix is formed from two polynucleotide strands.
 - Q9 Name the sugar in RNA.
 - Q10 Name the four possible bases in RNA.
 - Q11 Describe three differences between DNA and RNA.
 - Q12 What caused many scientists to doubt that DNA carried the genetic code?

2. DNA Replication

DNA is able to replicate itself and it does so on a regular basis. Clever thing.

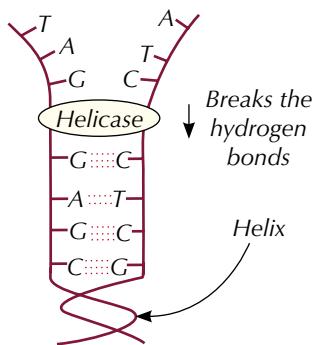
Why does DNA replicate?

DNA copies itself before cell division (see p. 86) so that each new cell has the full amount of DNA. The method is called semi-conservative replication because half of the strands in each new DNA molecule are from the original DNA molecule. This means that there's genetic continuity between generations of cells (i.e. the cells produced by cell division inherit their genes from their parent cells).

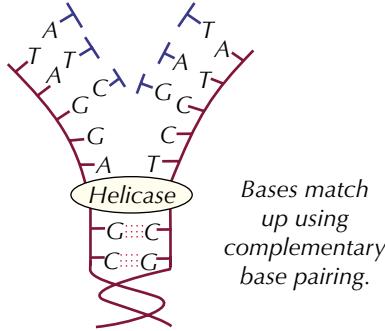
How is DNA replicated?

A DNA molecule has a paired base structure (see page 54), which makes it easy for DNA to copy itself. Here's how it works:

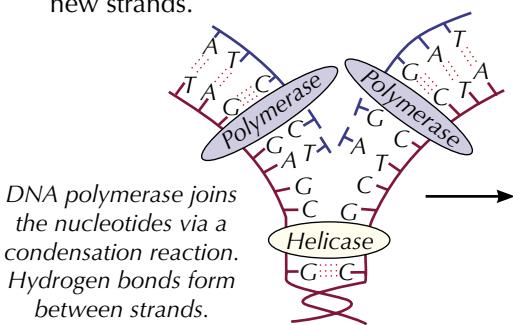
- 1 The enzyme **DNA helicase** breaks the hydrogen bonds between bases on the two polynucleotide DNA strands. This makes the helix unwind to form two single strands.



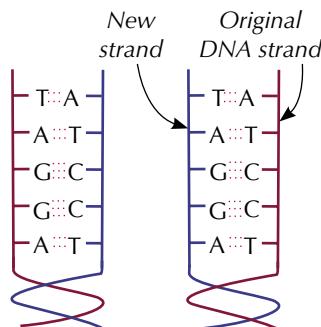
- 2 Each original single strand acts as a template for a new strand. Complementary base pairing means that free-floating DNA nucleotides are attracted to their complementary exposed bases on each original template strand — A with T and C with G.



- 3 Condensation reactions join the nucleotides of the new strand together — catalysed by the enzyme **DNA polymerase**. Hydrogen bonds form between the bases on the original and new strands.



Each new DNA molecule contains one strand from the original DNA molecule and one new strand.



Learning Objectives:

- Understand that the semi-conservative replication of DNA ensures genetic continuity between generations of cells.
- Understand the process of semi-conservative replication of DNA in terms of:
 - unwinding of the double helix,
 - the breakage of hydrogen bonds between complementary bases in the polynucleotide strands,
 - the role of DNA helicase in unwinding DNA and breaking its hydrogen bonds,
 - attraction of new DNA nucleotides to exposed bases on template strands and base pairing,
 - the role of DNA polymerase in the condensation reaction that joins adjacent nucleotides.
- Be able to evaluate the work of scientists in validating the Watson-Crick model of DNA replication.

Specification Reference 3.1.5.2

Exam Tip

If you're asked to describe the process of semi-conservative replication in the exam, you need to make sure you do it in the correct order or you won't get all the marks.

The action of DNA polymerase

Each end of a DNA strand is slightly different in its structure. One end is called the 3' (pronounced 'three prime') end and one end is called the 5' (five prime) end.

During DNA replication the active site of DNA polymerase is only complementary to the 3' end of the newly forming DNA strand — so the enzyme can only add nucleotides to the new strand at the 3' end. This means that the new strand is made in a 5' to 3' direction and that DNA polymerase moves down the template strand in a 3' to 5' direction — see Figure 1.

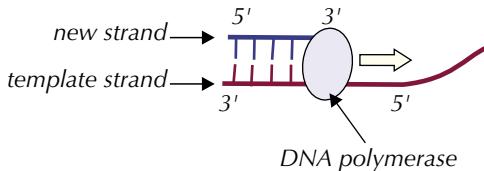


Figure 1: Detail of DNA polymerase action.

Because the strands in the double helix are antiparallel, the DNA polymerase working on one of the template strands moves in the opposite direction to the DNA polymerase working on the other template strand — see Figure 2.

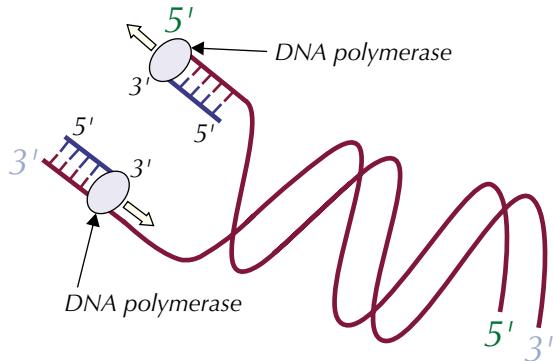
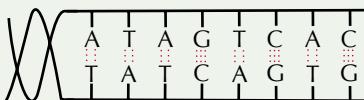


Figure 2: DNA polymerase working on a double stranded DNA molecule.

Practice Questions — Application

Tip: Have a look back at page 54 if you're struggling to remember which bases pair up with each other.

Q1 The diagram below shows a molecule of DNA.



Draw the original and replicated strands after semi-conservative replication.

Q2 The diagram below shows a template strand of DNA.

Give the sequence of the new strand that would be synthesised by the action of DNA polymerase. Write the sequence in the order that the bases would be added to the strand.



Evidence for semi-conservative replication

You might remember from page 56 that Watson and Crick determined the structure of DNA. They also came up with the theory of semi-conservative DNA replication.

However, it wasn't until Meselson and Stahl's experiment a few years later that this theory was validated. Before that, people were unsure whether DNA replication was semi-conservative or conservative. If the method was conservative, the original DNA strands would stay together and the new DNA molecules would contain two new strands.

Meselson and Stahl's experiment

Meselson and Stahl showed DNA is replicated using the semi-conservative method. Their experiment used two isotopes of nitrogen (DNA contains nitrogen) — heavy nitrogen (^{15}N) and light nitrogen (^{14}N).

1. Two samples of bacteria were grown for many generations — one in a nutrient broth containing light nitrogen, and one in a broth with heavy nitrogen. As the bacteria reproduced, they took up nitrogen from the broth to help make nucleotides for new DNA. So the nitrogen gradually became part of the bacteria's DNA.
2. A sample of DNA was taken from each batch of bacteria, and spun in a centrifuge. The DNA from the heavy nitrogen bacteria settled lower down the centrifuge tube than the DNA from the light nitrogen bacteria — because it's heavier (see Figure 3).

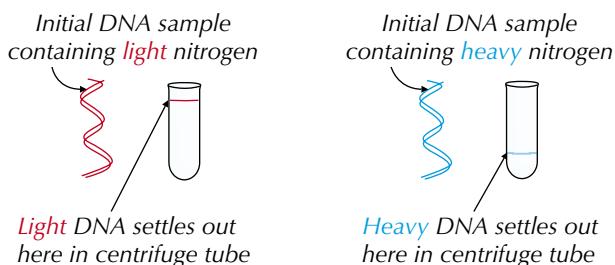


Figure 3: Diagram to show the results of steps 1 and 2 of the Meselson and Stahl experiment.

3. Then the bacteria grown in the heavy nitrogen broth were taken out and put in a broth containing only light nitrogen. The bacteria were left for one round of DNA replication, and then another DNA sample was taken out and spun in the centrifuge — see Figure 5.



Figure 5: Diagram to show step 3 of the Meselson and Stahl experiment.

4. If replication was conservative, the original heavy DNA, which would still be together, would settle at the bottom and the new light DNA would settle at the top — see Figure 6.
5. If replication was semi-conservative, the new bacterial DNA molecules would contain one strand of the old DNA containing heavy nitrogen and one strand of new DNA containing light nitrogen. So the DNA would settle out between where the light nitrogen DNA settled out and where the heavy nitrogen DNA settled out — see Figure 6.

Tip: Isotopes are different forms of the same element.

Tip: There's more on how centrifuges work on page 84.



Figure 4: Liquid growth medium or broth before bacteria are added.

Exam Tip

Remember, in the exam you might be asked about an experiment you're not familiar with. Don't panic though, just read it through and make sure you understand it before applying your knowledge to answer the questions on it.

DNA from bacteria that replicated once in *light* nitrogen broth

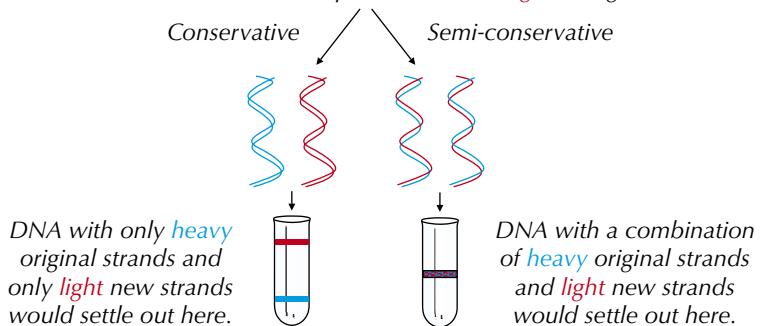


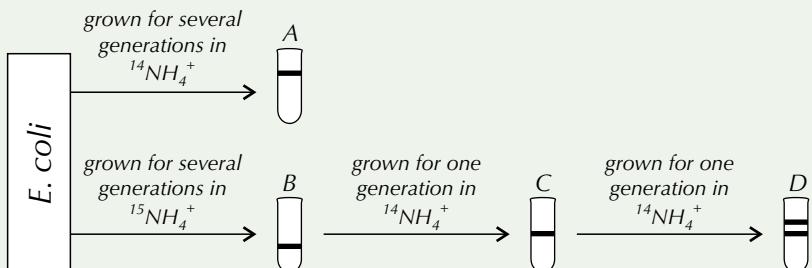
Figure 6: Diagram to show the possible results of step 3 of the Meselson and Stahl experiment.

6. As it turned out, the DNA settled out in the middle, showing that the DNA molecules contained a mixture of heavy and light nitrogen. The bacterial DNA had replicated semi-conservatively in the light nitrogen.

Once Meselson and Stahl had confirmed that DNA replication in bacteria was semi-conservative, other scientists carried out experiments to show that it was the universal method for DNA replication in all living things.

Practice Question — Application

- Q1 A scientist performed an experiment to demonstrate that DNA replicates in a semi-conservative way. As shown in the diagram, the scientist grew *E. coli* in nutrient broths containing either $^{15}\text{NH}_4^+$ (a source of heavy nitrogen) or $^{14}\text{NH}_4^+$ (a source of light nitrogen). At each stage of the experiment a DNA sample was extracted from the bacterial populations and spun in a centrifuge. The results are shown in the tubes on the diagram.



- a) Why were the results of centrifugation different in tubes A and B?
- b) Describe and explain the results seen in tube C.
- c) Describe and suggest reasons for the results seen in tube D.

Practice Questions — Fact Recall

- Q1 DNA is copied by semi-conservative replication of DNA. What is meant by this?
- Q2 Name the two enzymes involved in DNA replication.
- Q3 Describe the first stage of DNA replication, in which two strands of DNA are separated.
- Q4 Describe the second stage of DNA replication, where the single strands of DNA act as templates.

3. ATP

Energy is required for all life processes. This means that being able to store and release energy is really important for plants and animals.

Why is energy important?

Plant and animal cells need energy for biological processes to occur.

Examples

- Plants need energy for things like active transport (e.g. to transport solutes from their leaves — see p. 196), DNA replication (see p. 57), cell division (see p. 86) and protein synthesis (see p. 206).
- Animals need energy for things like active transport (e.g. to absorb glucose from the ileum epithelium into the bloodstream — see p. 111), DNA replication, cell division and protein synthesis.

Adenosine triphosphate (ATP)

Plant and animal cells release energy from glucose — this process is called respiration. A cell can't get its energy directly from glucose. So, in respiration, the energy released from glucose is used to make ATP (adenosine triphosphate).

ATP is made from the nucleotide base adenine, combined with a ribose sugar and three phosphate groups (see Figure 1). It's what's known as a nucleotide derivative because it's a modified form of a nucleotide.

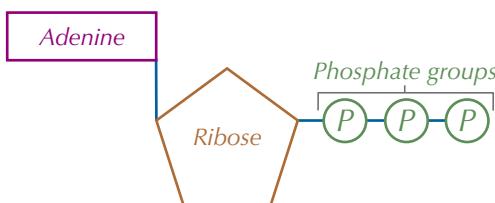


Figure 1: The structure of ATP.

Once made, ATP diffuses to the part of the cell that needs energy. The energy in ATP is stored in high energy bonds between the phosphate groups. It's released via hydrolysis reactions (see below).

Making and using ATP

When energy is needed by a cell, ATP is broken down into ADP (adenosine diphosphate) and P_i (inorganic phosphate). This is a **hydrolysis** reaction. A phosphate bond is broken and energy is released. The reaction is catalysed by the enzyme **ATP hydrolase** (see Figure 2).

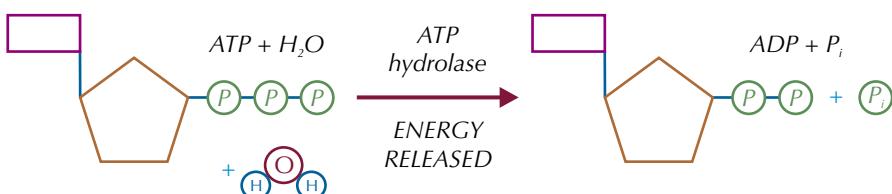


Figure 2: The breakdown of ATP — a hydrolysis reaction.

Learning Objectives:

- Understand that a single molecule of adenosine triphosphate (ATP) is a nucleotide derivative and is formed from a molecule of ribose, a molecule of adenine and three phosphate groups.
- Know that the hydrolysis of ATP to adenosine diphosphate (ADP) and an inorganic phosphate group (P_i) is catalysed by the enzyme ATP hydrolase.
- Understand that the hydrolysis of ATP can be coupled to energy-requiring reactions within cells.
- Know that the inorganic phosphate released during the hydrolysis of ATP can be used to phosphorylate other compounds, often making them more reactive.
- Know that ATP is resynthesised by the condensation of ADP and P_i.
- Understand that this reaction is catalysed by the enzyme ATP synthase during photosynthesis, or during respiration.

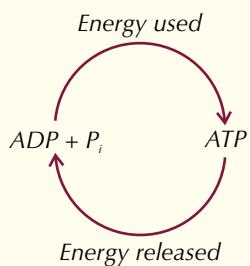
Specification Reference 3.1.6

Tip: Take a look at page 53 for a reminder about nucleotide structure.

Tip: Inorganic phosphate (P_i) is just the fancy name for a single phosphate.

Tip: Adenosine diphosphate has two phosphates. Adenosine triphosphate has three phosphates.

Tip: In a cell there's a constant cycle between ADP and P_i , and ATP. This allows energy to be stored and released as it's needed.



Tip: For more about how hydrolysis and condensation reactions work see pages 21-22.

Tip: It's important to remember that ATP isn't energy — it's a store of energy. Energy is used to make ATP, then it's released when ATP is hydrolysed to ADP and P_i .

ATP hydrolysis can be 'coupled' to other energy-requiring reactions in the cell — this means the energy released can be used directly to make the coupled reaction happen, rather than being lost as heat.

The released inorganic phosphate can also be put to use — it can be added to another compound (this is known as **phosphorylation**), which often makes the compound more reactive.

ATP can be resynthesised in a condensation reaction between ADP and P_i . This happens during both respiration and photosynthesis, and is catalysed by the enzyme **ATP synthase** (see Figure 3).

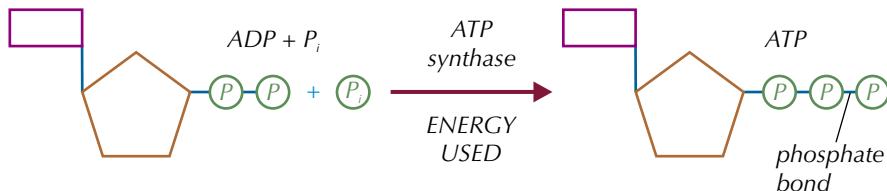


Figure 3: The synthesis of ATP — a condensation reaction.

Practice Questions — Application

- Q1 In addition to ADP and ATP, cells can also contain a molecule called AMP (adenosine monophosphate). Suggest what the structure of this molecule is.
- Q2 The movement of calcium ions across a cell membrane can occur via the energy-requiring process of active transport. This movement of calcium ions is coupled to the breakdown of ATP. Suggest why.

Practice Questions — Fact Recall

- Q1 What does 'ATP' stand for?
- Q2 Describe the structure of a molecule of ATP.
- Q3
 - a) What is ATP broken down into?
 - b) By what type of reaction is ATP broken down?
 - c) What enzyme catalyses the breakdown of ATP?
- Q4
 - a) How can inorganic phosphate (released by the breakdown of ATP) be used?
 - b) Give the abbreviation that can be used for inorganic phosphate.
- Q5 ATP can be reformed by the addition of an inorganic phosphate to ADP.
 - a) What type of reaction is this?
 - b) Give an example of a process during which this reaction takes place.

4. Water

Water is essential for life. The next few pages will show you what it is about water that makes it so important.

The importance of water

Water is vital to living organisms. It makes up about 80% of a cell's contents and has loads of important functions, inside and outside cells:

- Water is a metabolite in loads of important metabolic reactions, including condensation and hydrolysis reactions (see pages 21-22).
- Water is a solvent, which means some substances dissolve in it. Most metabolic reactions take place in solution (e.g. in the cytoplasm of eukaryotic and prokaryotic cells) so water's pretty essential.
- Water helps with temperature control because it has a high latent heat of vaporisation and a high specific heat capacity (see p. 65).
- Water molecules are very cohesive (they stick together), which helps water transport in plants (see p. 191) as well as transport in other organisms.

Structure of water

To understand the structure of water, you need to know a bit about the chemistry involved in holding water molecules together.

Polarity of water

A molecule of water (H_2O) is one atom of oxygen (O) joined to two atoms of hydrogen (H₂) by shared electrons — see Figure 1.

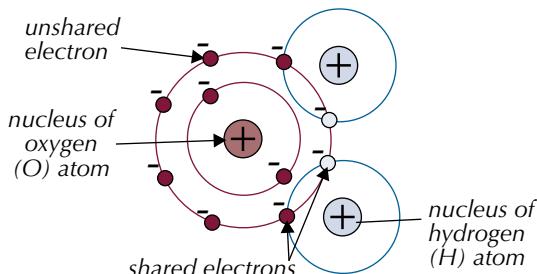


Figure 1: The structure of a water molecule.

Because the shared negative hydrogen electrons are pulled towards the oxygen atom, the other side of each hydrogen atom is left with a slight positive charge ($\delta+$). The unshared negative electrons on the oxygen atom give it a slight negative charge ($\delta-$). This makes water a polar molecule — it has a slight (partial) negative charge on one side and a slight (partial) positive charge on the other (see Figure 2).

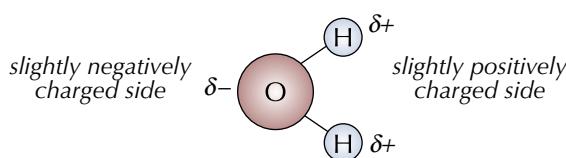


Figure 2: The slight charges on a water molecule.

Learning Objectives:

- Understand that water is a major component of cells. It has several properties that are important in biology. In particular, water:
 - is a metabolite in many metabolic reactions, including condensation and hydrolysis reactions,
 - is an important solvent in which metabolic reactions occur,
 - has a relatively large latent heat of vaporisation, providing a cooling effect with little loss of water through evaporation,
 - has a relatively high heat capacity, buffering changes in temperature,
 - has strong cohesion between water molecules; this supports columns of water in the tube-like transport cells of plants and produces surface tension where water meets air.

Specification Reference 3.1.7

Tip: We know that water, as the most common component of cells, is really important to living organisms. That's why our search for other life in the universe involves searching for liquid water.

Tip: ' $\delta+$ ' is pronounced 'delta positive' and ' $\delta-$ ' is 'delta negative'.

Exam Tip

If you're asked to draw water molecules in the exam, make sure you draw the hydrogen bonds as dashed lines and include the partial charges ($\delta+$ or $\delta-$) on all the atoms.

Tip: A metabolic reaction is a chemical reaction that happens in a living organism to keep the organism alive. A metabolite is a substance involved in a metabolic reaction.

Tip: Most biological reactions take place in solution, so water's pretty essential.

Tip: Remember — a molecule is polar if it has a slightly negatively charged side and a slightly positively charged side.

Tip: Polar molecules, such as glucose, dissolve in water because hydrogen bonds form between them and the water molecules.

Exam Tip

If you're asked about how a particular ion dissolves in water, don't get put off by the ion itself — just figure out if it's positively charged or negatively charged.

Hydrogen bonding

Hydrogen bonds are weak bonds between a slightly positively charged hydrogen atom in one molecule and a slightly negatively charged atom in another molecule. Hydrogen bonds form between water molecules because the slightly negatively charged oxygen atoms of water attract the slightly positively charged hydrogen atoms of other water molecules. This hydrogen bonding gives water some of its useful properties.

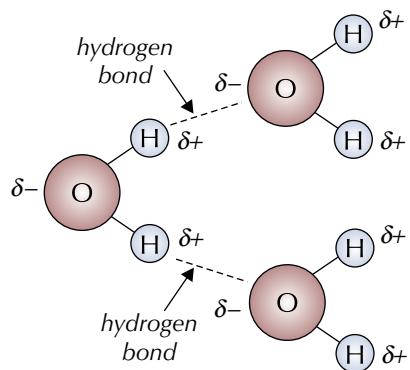


Figure 3: Diagram showing how hydrogen bonds hold water molecules together.

Properties of water

Here's a bit more about each of the useful properties of water that you need to learn for your exam.

Important metabolite

Many metabolic reactions involve a condensation or hydrolysis reaction. A hydrolysis reaction requires a molecule of water to break a bond. A condensation reaction releases a molecule of water as a new bond is formed. For example, amino acids are joined together to make polypeptides (proteins) by condensation reactions (see page 33). Energy from ATP is released through a hydrolysis reaction (see page 61).

Good solvent

A lot of important substances in biological reactions are ionic (like salt, for example). This means they're made from one positively charged atom or molecule and one negatively charged atom or molecule (e.g. salt is made from a positive sodium ion and a negative chloride ion).

Because water is polar, the slightly positively charged end of a water molecule will be attracted to the negative ion, and the slightly negatively charged end of a water molecule will be attracted to the positive ion. This means the ions will get totally surrounded by water molecules — in other words, they'll dissolve (see Figure 4). So water's polarity makes it useful as a solvent (a substance capable of dissolving another substance). This means living organisms can take up useful substances (e.g. mineral ions) dissolved in water and these dissolved substances can be transported around the organism's body.

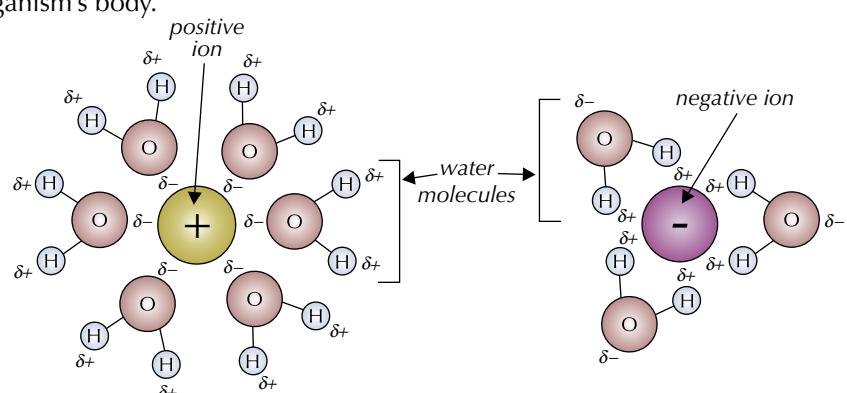


Figure 4: A positive ion (left) and a negative ion (right) dissolved in water.

High latent heat of vaporisation

Water evaporates (vaporises) when the hydrogen bonds holding water molecules together are broken. This allows the water molecules on the surface of the water to escape into the air as a gas. It takes a lot of energy (heat) to break the hydrogen bonds between water molecules, so a lot of energy is used up when water evaporates. This means water has a high latent heat of vaporisation — lots of heat is used to change it from a liquid to a gas.

This is useful for living organisms because it means they can use water loss through evaporation to cool down without losing too much water. When water evaporates it carries away heat energy from a surface, which cools the surface and helps to lower the temperature (e.g. when humans sweat to cool down).

Tip: Latent heat is the heat energy that's needed to change a substance from one state to another, e.g. from a liquid to a gas.

Can buffer (resist) changes in temperature

Hydrogen bonds give water a high **specific heat capacity** — this is the energy needed to raise the temperature of 1 gram of a substance by 1 °C. When water is heated, a lot of the heat energy is used to break the hydrogen bonds between the water molecules. This means there is less heat energy available to actually increase the temperature of the water. So water has a high specific heat capacity — it takes a lot of energy to heat it up.

This is useful for living organisms because it means that water doesn't experience rapid temperature changes. This makes water a good habitat because the temperature under water is likely to be more stable than on land. The water inside organisms also remains at a fairly stable temperature — helping them to maintain a constant internal body temperature.

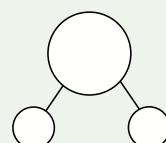
Tip: Enzyme activity is affected by temperature (see page 40). Some important biological processes need enzymes to work (e.g. digestion and respiration) — these may not work properly if the organism's temperature is not kept fairly stable.

Very cohesive

Cohesion is the attraction between molecules of the same type (e.g. two water molecules). Water molecules are very cohesive (they tend to stick together) because they're polar. Strong cohesion helps water to flow, making it great for transporting substances. For example, it's how water travels in columns up the xylem (tube-like transport cells) in plants (see p. 191). Strong cohesion also means that water has a high surface tension when it comes into contact with air. This is the reason why sweat forms droplets, which evaporate from the skin to cool an organism down. It's also the reason that pond skaters, and some other insects, can 'walk' on the surface of a pond.

Practice Questions — Fact Recall

- Q1 Name two reactions that water is involved in.
- Q2 Why is water classed as a polar molecule?
- Q3 Label this diagram of a water molecule → showing the name and charge on each atom.
- Q4 What is a hydrogen bond?
- Q5 What is a metabolite?
- Q6 What makes water useful as a solvent?
- Q7 Water has a high latent heat of vaporisation. What does this mean?
- Q8 Explain why water has a high specific heat capacity.
- Q9 a) What is cohesion?
b) Why is cohesion between water molecules important in plants?



Learning Objectives:

- Know that inorganic ions occur in solution in the cytoplasm and body fluids of organisms, some in high concentrations and others in very low concentrations.
- Understand that each type of ion has a specific role, depending on its properties.
- Be able to recognise the role of ions in the following topics:
 - iron ions as a component of haemoglobin,
 - hydrogen ions and pH,
 - sodium ions in the co-transport of glucose and amino acids,
 - phosphate ions as components of DNA and of ATP.

Specification Reference 3.1.8

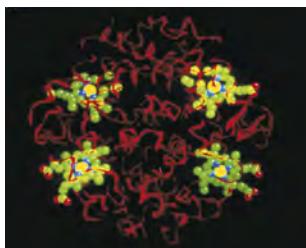


Figure 1: A computer model of a haemoglobin molecule.

There's an iron atom (the yellow sphere) in each of the four polypeptide chains.

5. Inorganic Ions

Ions might not be the first thing you think about when you think of biological molecules but they have some very important roles in organisms.

What are ions?

An **ion** is an atom (or group of atoms) that has an electric charge. An ion with a positive charge is called a cation.

Examples

Na⁺ — this is a sodium ion, it has a charge of +1.

Ca²⁺ — this is a calcium ion, it has a charge of +2.

An ion with a negative charge is called an anion.

Examples

Cl⁻ — this is a chlorine ion, it has a charge of -1.

PO₄³⁻ — this is a phosphate ion, it has a charge of -3.

Inorganic ions

An inorganic ion is one which doesn't contain carbon (although there are a few exceptions to this rule). There are inorganic ions, in solution, in the cytoplasm of cells and in the body fluids of organisms. Each ion has a specific role, depending on its properties. An ion's role determines whether it is found in high or low concentrations.

Examples

Iron ions in haemoglobin

Haemoglobin is a large protein that carries oxygen around the body, in the red blood cells. It's made up of four different polypeptide chains, each with an iron ion (Fe²⁺) in the centre. It's the Fe²⁺ that actually binds to the oxygen in haemoglobin — so it's a pretty key component. When oxygen is bound, the Fe²⁺ ion temporarily becomes an Fe³⁺ ion, until oxygen is released.

Hydrogen ions

pH is calculated based on the concentration of hydrogen ions (H⁺) in the environment. The more H⁺ present, the lower the pH (and the more acidic the environment). Enzyme-controlled reactions are all affected by pH.

Sodium ions

Glucose and amino acids need a bit of help crossing cell membranes. A molecule of glucose or an amino acid can be transported into a cell (across the cell-surface membrane) alongside sodium ions (Na⁺). This is known as co-transport (see pages 110 and 111 for more).

Phosphate ions

When a phosphate ion (PO₄³⁻) is attached to another molecule, it's known as a phosphate group. DNA, RNA and ATP all contain phosphate groups. It's the bonds between phosphate groups that store energy in ATP (see page 61). The phosphate groups in DNA and RNA allow nucleotides to join up to form the polynucleotides (see p. 53).

Practice Questions — Fact Recall

- Q1 What is an inorganic ion?
- Q2 Where do inorganic ions occur?
- Q3 What determines an ion's specific role?
- Q4 a) What ions are part of haemoglobin molecules?
b) What is the role of these ions in haemoglobin?
- Q5 Name the ion that is linked to pH.
- Q6 a) Which type of ion is involved in moving glucose and amino acids across cell membranes?
b) What is the name of this process?

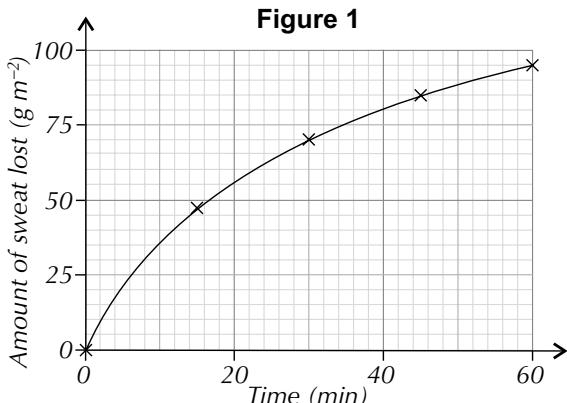
Section Summary

Make sure you know:

- How DNA and RNA are important molecules that carry information.
- That ribosomes are formed from RNA and proteins.
- The general structure of a nucleotide, and how the structure of DNA and RNA nucleotides differ.
- That DNA and RNA are both nucleotide polymers that contain phosphodiester bonds between nucleotides as a result of condensation reactions.
- That there are differences between the overall DNA and RNA molecule structures — DNA forms a double helix with hydrogen bonds between two polynucleotide strands as a result of complementary base pairing, whereas molecules of RNA form single polynucleotide strands and are short in length compared to DNA.
- Why, in the past, scientists doubted that DNA was capable of carrying the genetic code.
- Why DNA replication is semi-conservative and how the process takes place — the hydrogen bonds between the bases on polynucleotide strands are broken by DNA helicase, free DNA nucleotides are attracted to their complementary exposed bases, and the nucleotides are joined together by DNA polymerase to form a DNA molecule with one new strand and one original strand.
- How to evaluate the work of scientists in validating the Watson-Crick model of DNA replication, e.g. Meselson and Stahl's experiment (to show semi-conservative replication in DNA).
- That ATP is a modified nucleotide composed of adenine, a ribose sugar and three phosphate groups.
- That ATP is hydrolysed to ADP and an inorganic phosphate group (P_i) by the enzyme ATP hydrolase and that the energy released from this hydrolysis reaction can be used directly in a coupled reaction.
- That the P_i released from ATP hydrolysis can be added to another compound (by phosphorylation) which often makes the compound more reactive.
- That ATP can be resynthesised from ADP and P_i in a condensation reaction that's catalysed by ATP synthase — this reaction occurs during photosynthesis and respiration.
- That water makes up 80% of a cell's contents and has lots of important properties such as: it functions as a metabolite, it's a good solvent, it has a large latent heat of vaporisation allowing organisms to lose heat through evaporation, it has a high specific heat capacity and so can buffer changes in temperature, and it's a very cohesive substance.
- That inorganic ions are ions that don't contain carbon and that they occur, in solution, in the cytoplasms of cells and in the body fluids of organisms.
- That inorganic ions have specific roles depending on their properties and that you know the roles of iron, hydrogen, sodium and phosphate ions in the body.

Exam-style Questions

- 1 Inside the cell, the mitochondrion produces ATP.
Molecules of ATP contain three phosphate groups.
- 1.1 Describe the rest of the structure of ATP. (2 marks)
- 1.2 Name the enzyme that catalyses the formation of ATP. (1 mark)
- 1.3 Name **one** other molecule that contains a phosphate ion. (1 mark)
- ATP hydrolysis is coupled to the action of DNA helicases in unwinding a DNA molecule during DNA replication.
- 1.4 Describe the reaction of ATP hydrolysis. (3 marks)
- 1.5 Suggest why ATP hydrolysis is coupled to the action of DNA helicase. (2 marks)
- 2 When humans exercise vigorously they lose water from their bodies in sweat.
- 2.1 Name the property of water that enables sweating to have a cooling effect on the body during exercise and explain how it has this effect. (3 marks)
- Sweat contains ions, such as sodium ions (Na^+), dissolved in water.
- 2.2 Describe how sodium ions dissolve in water. (3 marks)
- 2.3 Give **one** use of sodium ions in the body. (1 mark)
- Figure 1** shows the amount of sweat lost by a person over the course of one hour of exercise.
- 2.4 How much sweat had the person lost after 14 minutes? (1 mark)
- 2.5 Calculate the person's sweat rate at 20 minutes. (2 marks)



- 3** DNA replication is the process by which DNA copies itself. The process can be divided into a number of stages which are listed in **Table 1** below.

Table 1

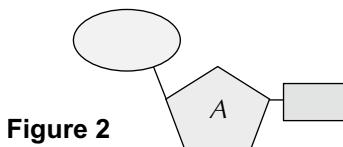
| Stage | Description |
|----------|---|
| A | Adjacent DNA nucleotides on each new DNA strand are joined together by an enzyme. Hydrogen bonds form between the bases on the original and new strands. |
| B | DNA helicase attaches to the DNA molecule and breaks the hydrogen bonds between the bases on the two polynucleotide strands. The DNA unwinds to form two strands. |
| C | Free-floating DNA nucleotides are attracted to exposed bases on each template strand. |
| D | Two DNA molecules are produced. Each one contains one strand from the original DNA molecule (the template strand) and one new strand. |

- 3.1** The stages shown above are not listed in the correct order.
List the stages in the correct order (1 mark)
- 3.2** Name the enzyme involved in Stage **A**. (1 mark)
- 3.3** In Stage **A**, name the type of reaction that joins adjacent DNA nucleotides together. (1 mark)
- 3.4** In Stage **C**, free-floating nucleotides are attracted to exposed bases on the template strand via complementary base pairing. Explain what happens in complementary base pairing. (2 marks)

- 4** DNA and RNA are both polynucleotides but have different functions.

- 4.1** Describe the difference between the functions of the two molecules. (1 mark)

Figure 2 below shows a general nucleotide structure.



- 4.2** How is part **A** different in a DNA nucleotide compared to an RNA nucleotide? (1 mark)
- 4.3** Describe **two** other differences between the structures of the polynucleotides DNA and RNA. (2 marks)

Learning Objectives:

- Know the structure of eukaryotic cells, restricted to the structure and function of:
 - the cell-surface membrane
 - the nucleus (containing chromosomes consisting of protein-bound linear DNA and one or more nucleoli)
 - mitochondria
 - chloroplasts
 - Golgi apparatus and Golgi vesicles
 - lysosomes (a Golgi vesicle containing lysozymes)
 - ribosomes
 - rough endoplasmic reticulum and smooth endoplasmic reticulum
 - the cell wall
 - the cell vacuole (of plant cells only).
- Understand that in complex multicellular organisms, eukaryotic cells become specialised for specific functions.
- Be able to apply your knowledge of these features in explaining adaptations of eukaryotic cells.
- Know that specialised cells are organised into tissues, tissues into organs and organs into systems.

Specification Reference 3.2.1.1

1. Eukaryotic Cells and Organelles

No doubt you learnt about cell structure at GCSE, but there's a lot more to it at this level — as you're about to find out...

Eukaryotes and prokaryotes

All living organisms are made of cells, which have the same basic features in common. This suggests that all living things evolved from the same common ancestor (see page 21 for more).

There are two main types of organism — eukaryotes and prokaryotes. Prokaryotic organisms are **prokaryotic cells** (i.e. they're single-celled organisms). Eukaryotic organisms are made up of **eukaryotic cells**. Both types of cells contain organelles (see below). Eukaryotic cells are complex. Prokaryotic cells are smaller and simpler. There's more on prokaryotic cells on page 77.

Organelles

Organelles are parts of cells. Each one has a specific function. If you examine a cell through an electron microscope (see page 82) you can see its organelles and the internal structure of most of them. Everything you need to know about eukaryotic cell organelles is covered over the next few pages.

Eukaryotic cells

Eukaryotic cells are generally a bit more complicated than prokaryotic cells and have more organelles. Animal, plant, algal and fungal cells are all eukaryotic. You've probably been looking at animal and plant cell diagrams for years, so hopefully you'll be familiar with some of the bits and pieces...

Animal cells

Figure 1 shows the organelles found in a typical animal cell. You can compare these to the ones found in a typical plant cell on the next page.

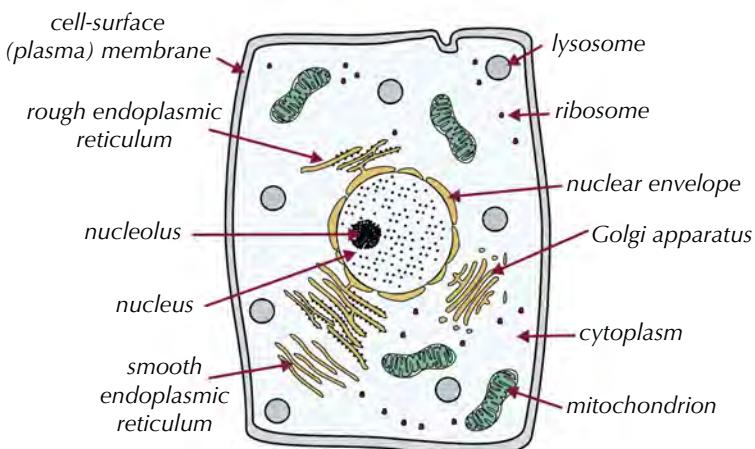


Figure 1: The structure of a typical animal cell.

Plant cells

Plant cells have the same organelles as animal cells, but with a few added extras:

- a cellulose cell wall with plasmodesmata ('channels' for exchanging substances between adjacent cells),
- a vacuole (fluid-filled compartment),
- and of course good old chloroplasts (the organelles involved in photosynthesis).

These organelles are all shown in Figure 2.

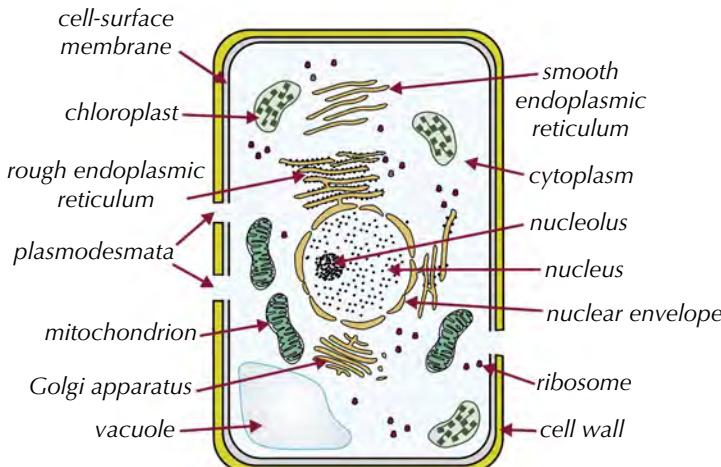


Figure 2: The structure of a typical plant cell.

Exam Tip

In the exam, you might be shown electron micrographs of different types of cell, as well as diagrams like the ones here. Make sure you know all the distinguishing features for each cell type.

Tip: You might also see starch grains in plant cells, although they're not organelles. Plants use starch grains to store excess sugars.

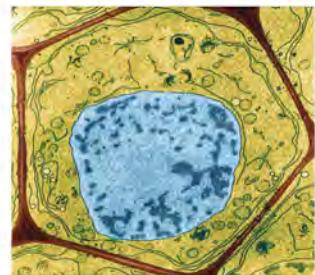


Figure 3: An electron micrograph of a plant cell.

The cell walls appear red/brown and the nucleus appears blue.

Algal cells

Algae carry out photosynthesis, like plants, but unlike plants they can be unicellular (e.g. *Chlorella*) or multicellular (e.g. seaweed). Figure 4 shows some of the features of an algal cell.

Algal cells are a lot like plant cells — they have all the same organelles, including a cellulose cell wall and chloroplasts. However, the chloroplasts in many algal cells are a different shape and size to plant chloroplasts. For example, some algae have one large chloroplast rather than several smaller chloroplasts.

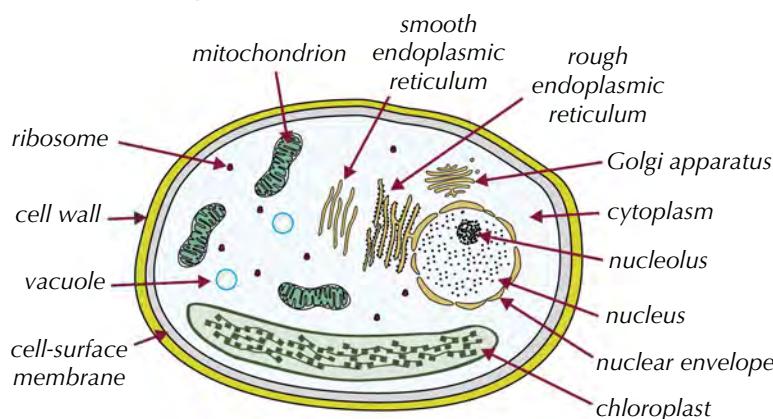


Figure 4: Structural features of an algal cell.

Tip: There are lots of different types of animal, plant, algal and fungal cells and they won't all look exactly like the ones shown here or have exactly the same organelles (e.g. not all plant cells contain chloroplasts).

Fungal cells

Fungi can also be multicellular (e.g. mushrooms) or unicellular (e.g. yeast). Fungal cells (Figure 5) are also a lot like plant cells, but with two key differences:

- their cell walls are made of chitin, not cellulose.
- they don't have chloroplasts (because they don't photosynthesise).

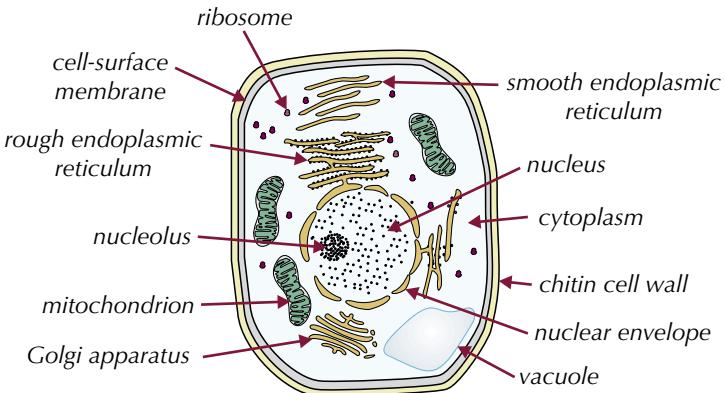


Figure 5: Structural features of a fungal cell.

Exam Tip

You could be asked to identify organelles seen under the microscope in your exam — so get learning the diagrams in this list. You need to be able to identify parts of the organelles' internal structures too.

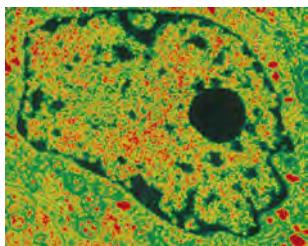


Figure 6: An electron micrograph of a nucleus, showing the nucleolus and nuclear envelope.

Tip: The plural of nucleus is nuclei and the plural of nucleolus is nucleoli. Weird.

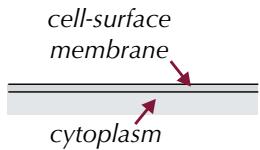
Functions of organelles

Here's a big list of organelles — you need to know the structure and function of them all. Sorry.

Cell-surface membrane (Also called the plasma membrane)

Description

The membrane found on the surface of animal cells and just inside the cell wall of other cells. It's made mainly of lipids and protein.



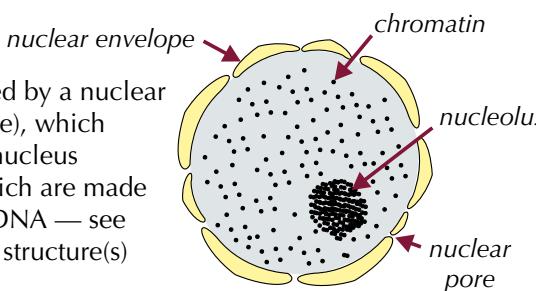
Function

Regulates the movement of substances into and out of the cell. It also has receptor molecules on it, which allow it to respond to chemicals like hormones. See pages 97-98 for more.

Nucleus

Description

A large organelle surrounded by a nuclear envelope (double membrane), which contains many pores. The nucleus contains **chromosomes** (which are made from protein-bound linear DNA — see page 203) and one or more structure(s) called a **nucleolus**.



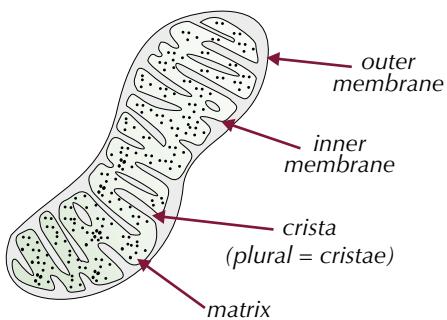
Function

The nucleus controls the cell's activities (by controlling the transcription of DNA — see pages 207-208). DNA contains instructions to make proteins. The pores allow substances (e.g. RNA) to move between the nucleus and the cytoplasm. The nucleolus makes ribosomes (see page 74).

Mitochondrion

Description

They're usually oval-shaped. They have a double membrane — the inner one is folded to form structures called cristae. Inside is the matrix, which contains enzymes involved in respiration.



Tip: The plural of mitochondrion is mitochondria.

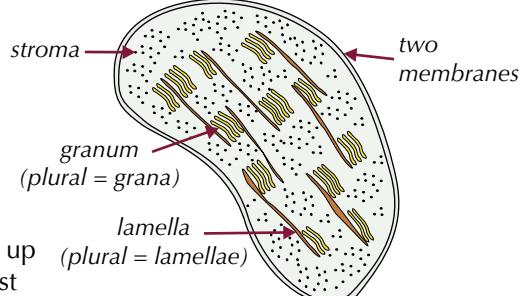
Function

The site of aerobic respiration. Aerobic respiration produces ATP — a common energy source in the cell. Mitochondria are found in large numbers in cells that are very active and require a lot of energy.

Chloroplast

Description

A small, flattened structure found in plant cells and algal cells. It's surrounded by a double membrane, and also has membranes inside called thylakoid membranes.



These membranes are stacked up in some parts of the chloroplast to form grana. Grana are linked together by lamellae — thin, flat pieces of thylakoid membrane.

Function

The site where photosynthesis takes place. Some parts of photosynthesis happen in the grana, and other parts happen in the stroma (a thick fluid found in chloroplasts).



Figure 7: An electron micrograph of a mitochondrion.

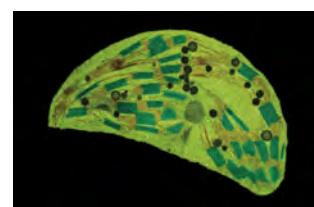
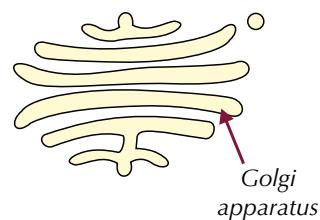


Figure 8: An electron micrograph of a chloroplast.

Golgi apparatus

Description

A group of fluid-filled membrane-bound flattened sacs. Vesicles (see below) are often seen at the edges of the sacs.



Function

It processes and packages new lipids and proteins. It also makes lysosomes (see next page).

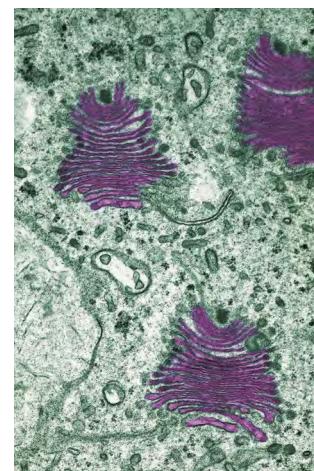
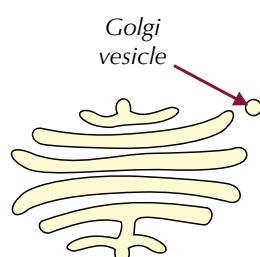


Figure 9: An electron micrograph of Golgi apparatus.

Golgi vesicle

Description

A small fluid-filled sac in the cytoplasm, surrounded by a membrane and produced by the Golgi apparatus.



Function

Stores lipids and proteins made by the Golgi apparatus and transports them out of the cell (via the cell-surface membrane).

Tip: Most eukaryotic organelles are surrounded by membranes, which sometimes causes confusion — don't make the mistake of thinking that a diagram of an organelle is a diagram of a whole cell. They're not cells — they're parts of cells.



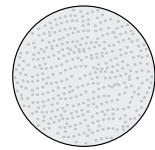
Figure 10: An electron micrograph showing SER (red-brown) and RER (blue).

Tip: Electron micrographs like the one above are produced in black and white. Any colour is added artificially afterwards.

Lysosome

Description

A round organelle surrounded by a membrane, with no clear internal structure. It's a type of Golgi vesicle.



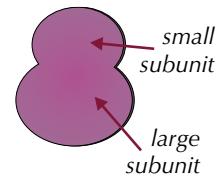
Function

Contains digestive enzymes called lysozymes. These are kept separate from the cytoplasm by the surrounding membrane, and can be used to digest invading cells or to break down worn out components of the cell.

Ribosome

Description

A very small organelle that floats free in the cytoplasm or is attached to the rough endoplasmic reticulum. It's made up of proteins and RNA (see page 55). It's not surrounded by a membrane.



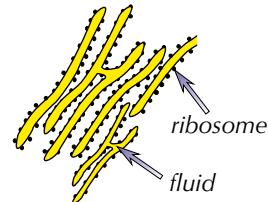
Function

The site where proteins are made.

Rough endoplasmic reticulum (RER)

Description

A system of membranes enclosing a fluid-filled space. The surface is covered with ribosomes.



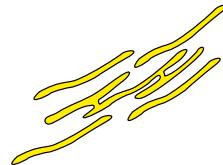
Function

Folds and processes proteins that have been made at the ribosomes.

Smooth endoplasmic reticulum (SER)

Description

Similar to rough endoplasmic reticulum, but with no ribosomes.



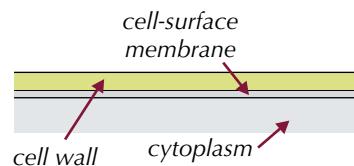
Function

Synthesises and processes lipids.

Cell wall

Description

A rigid structure that surrounds cells in plants, algae and fungi. In plants and algae it's made mainly of the carbohydrate cellulose. In fungi, it's made of chitin.



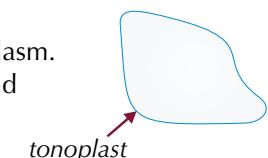
Function

Supports cells and prevents them from changing shape.

Cell vacuole (plants)

Description

A membrane-bound organelle found in the cytoplasm. It contains cell sap — a weak solution of sugar and salts. The surrounding membrane is called the tonoplast.



Function

Helps to maintain pressure inside the cell and keep the cell rigid. This stops plants wilting. It's also involved in the isolation of unwanted chemicals inside the cell.

Cell function and organelles

In multicellular eukaryotic organisms, cells become specialised to carry out specific functions.

A cell's structure (i.e. its shape and the organelles it contains) helps it to carry out its function — so depending on what job it does, a specialised cell can look very different to the cells you saw on pages 70-72.

In the exam, you might get a question where you need to apply your knowledge of organelles to explain why a specialised cell is particularly suited to its function. You'll need to think about what organelles the cell needs to do its job — e.g. if the cell uses a lot of energy, it'll need lots of mitochondria. If it makes a lot of proteins it'll need a lot of ribosomes.

Exam Tip

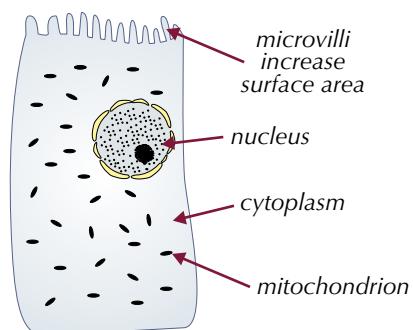
Never say mitochondria produce energy in the exam — they produce ATP or release energy (energy can't be made).

Examples

Epithelial cells

Epithelial cells in the small intestine are adapted to absorb food efficiently:

- The walls of the small intestine have lots of finger-like projections called villi. These increase surface area for absorption.
- The epithelial cells on the surface of the villi have folds in their cell-surface membranes, called **microvilli**. Microvilli increase surface area even more.
- They also have lots of mitochondria — to provide energy for the transport of digested food molecules into the cell.



Red blood cells

Red blood cells are adapted to carry oxygen around the body. They have no nucleus to make more room for the oxygen-carrying compound haemoglobin.

Sperm cells

Sperm cells contain a lot of mitochondria to provide the large amounts of energy they need to propel themselves towards an egg.

Exam Tip

In the exam they could throw any type of cell at you — don't panic if you haven't heard of it though, just focus on its function and you'll be able to figure out the organelles it needs to do its job.

Cell organisation

In multicellular eukaryotic organisms, specialised cells are grouped together to form **tissues**. A tissue is a group of cells working together to perform a particular function. Different tissues work together to form **organs**.

Different organs make up an **organ system**.

Example

Epithelial cells make up epithelial tissue. Epithelial tissue, muscular tissue and glandular tissue (which secretes chemicals) all work together to form the stomach — an organ. The stomach is part of the digestive system — this is an organ system made up of all the organs involved in the digestion and absorption of food (including the small intestine, large intestine and liver).

Practice Questions — Application

Q1 Figure 11 below shows a mitochondrion.

Name the parts labelled A-C.

Q2 Identify the organelle shown in Figure 12.

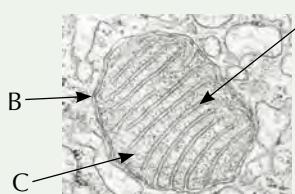


Figure 11



Figure 12

Tip: The mitochondrion here appears round rather than elongated like the one on page 73 because of the way the specimen was cut for the electron micrograph.

Q3 Below is a list of cell types and their function.

| Cell type | Function |
|------------------------------------|--|
| Cardiac muscle cells | Contraction of the heart. |
| Alveolar macrophage cells | To ingest and digest pathogens invading the lungs. |
| Beta cells in islets of Langerhans | To produce insulin (a protein). |
| Proximal tubule epithelial cells | To reabsorb useful molecules filtered out of the blood by the kidneys. |

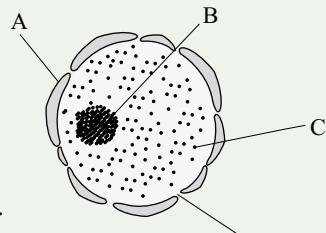
- a) Name one organelle you would expect to find a lot of in cardiac muscle cells. Give a reason for your answer.
- b) Suggest how alveolar macrophage cells are adapted to their function in terms of the organelles they contain.
- c) Name three organelles you would expect to find a lot of in beta cells in the islets of Langerhans.
- d) Suggest how proximal tubule epithelial cells are adapted to their function in terms of the organelles they contain.

Practice Questions — Fact Recall

Q1 Give two functions of the cell-surface membrane.

Q2 The diagram on the right is of a cell nucleus.

Name the structures labelled A-D.



Q3 Describe the function of the nucleus.

Q4 Describe the appearance of the Golgi apparatus.

Q5 Give one function of a lysosome.

Q6 What is the function of the smooth endoplasmic reticulum?

Q7 Explain the difference between a tissue and an organ.

Q8 What is an organ system?

2. Prokaryotic Cells and Viruses

Prokaryotic cells and viruses are different from eukaryotic cells — in fact, viruses aren't even cells. And you can't get much more different than that. You need to learn their structures and how they replicate.

Prokaryotic cell structure

Prokaryotes are single-celled organisms. Bacteria (like *E. coli*) are examples of prokaryotes.

You need to know the structure of a prokaryotic cell and what all the different organelles inside are for — see Figure 1. Prokaryotic cells are much smaller and simpler than eukaryotic cells — and they don't have any membrane-bound organelles (like a nucleus) in their cytoplasm.

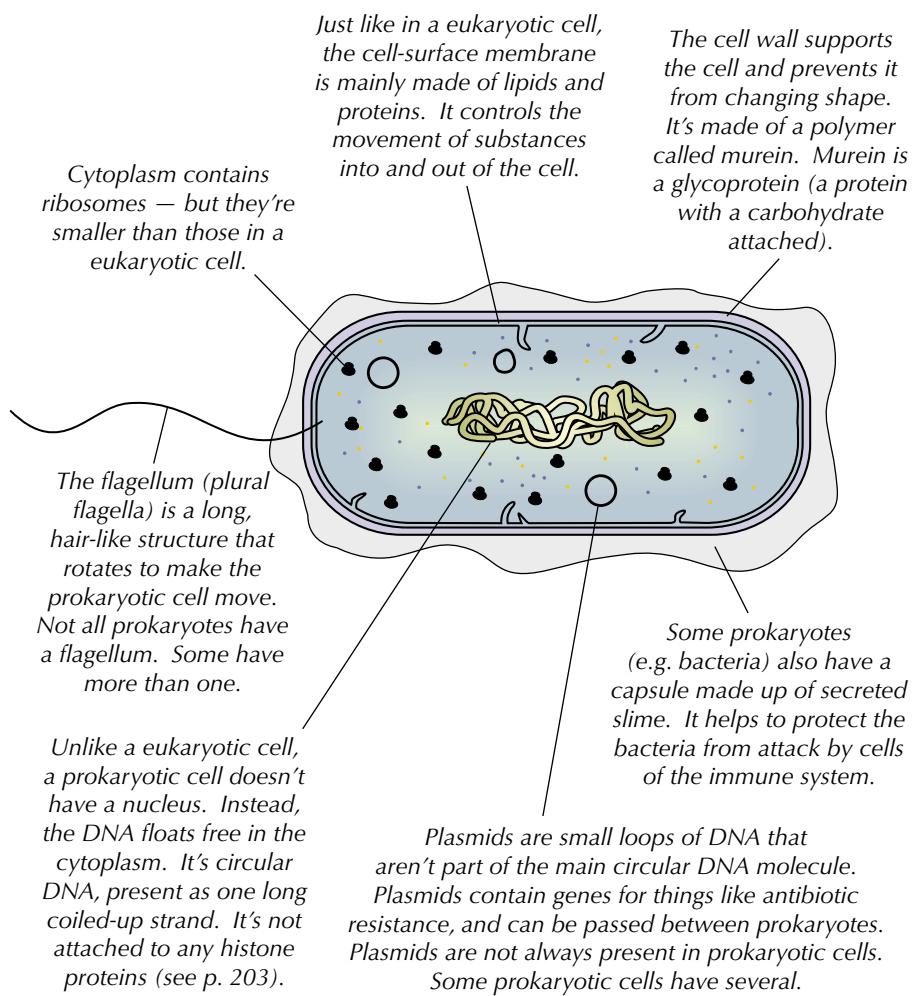


Figure 1: The structure of a prokaryotic cell.

Prokaryotic cells are extremely small — less than 2 µm in diameter (that's two millionths of a metre or 0.002 mm). Eukaryotic cells can be up to 50 times bigger (although that's still only around 0.1 mm).

Learning Objectives:

- Know that prokaryotic cells are much smaller than eukaryotic cells and differ from eukaryotic cells in having:
 - cytoplasm that lacks membrane-bound organelles
 - smaller ribosomes
 - no nucleus (instead they have a single circular DNA molecule that is free in the cytoplasm and is not associated with proteins)
 - a cell wall that contains murein (a glycoprotein).
- Know that prokaryotic cells may also have one or more plasmids, a capsule surrounding the cell and one or more flagella.
- Know that binary fission in prokaryotic cells involves replication of the circular DNA and plasmids and division of the cytoplasm to produce two daughter cells (each with a single copy of the circular DNA and a variable number of copies of plasmids).
- Recall that viruses are acellular and non-living.
- Know the structure of virus particles.
- Know that viruses do not undergo cell division. Following injection of their nucleic acid, the infected host cell replicates virus particles.

Specification Reference 3.2.1.2 and 3.2.2



Figure 2: An electron micrograph of the bacteria that cause cholera. The long 'tails' are flagella.



Figure 4: *E.coli* bacteria replicating by binary fission. The red blobs are DNA.

Tip: DNA and RNA are nucleic acids — see pages 53-55 for more.

Tip: There are lots of different viruses and they can look very different from each other. Some viruses (e.g. HIV) also have an envelope, which surrounds the capsid.

Prokaryotic cell replication

Prokaryotic cells replicate by a process called **binary fission**. In binary fission, the cell replicates (makes copies of) its genetic material, before physically splitting into two daughter cells.

The process of binary fission

Step 1

The circular DNA and plasmid(s) replicate. The main DNA loop is only replicated once, but plasmids can be replicated loads of times.

Step 2

The cell gets bigger and the DNA loops move to opposite 'poles' (ends) of the cell.

Step 3

The cytoplasm begins to divide (and new cell walls begin to form).

Step 4

The cytoplasm divides and two daughter cells are produced. Each daughter cell has one copy of the circular DNA, but can have a variable number of copies of the plasmid(s).

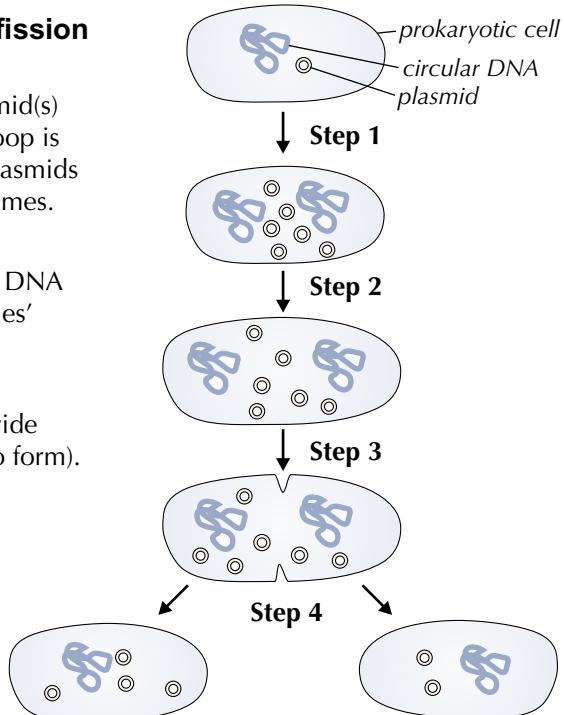


Figure 3: A prokaryotic cell undergoing binary fission.

Viruses

Viruses are **acellular** — they're not cells. In fact, viruses are just nucleic acids surrounded by protein — they're not even alive. Examples of viruses include HIV (which causes AIDS — see p. 132), influenza (which causes the flu) and rhinoviruses (which cause colds). All viruses invade and reproduce inside the cells of other organisms (see next page). These cells are known as **host cells**.

You need to learn the basic structure of a virus — see Figure 5.

Unlike bacteria, viruses have no cell-surface membrane, no cytoplasm and no ribosomes. They do have a protein coat, called a **capsid**, with **attachment proteins** sticking out from it. The attachment proteins let the virus cling onto a suitable host cell. Viruses are even smaller than bacteria — e.g. HIV is about 0.1 µm across.

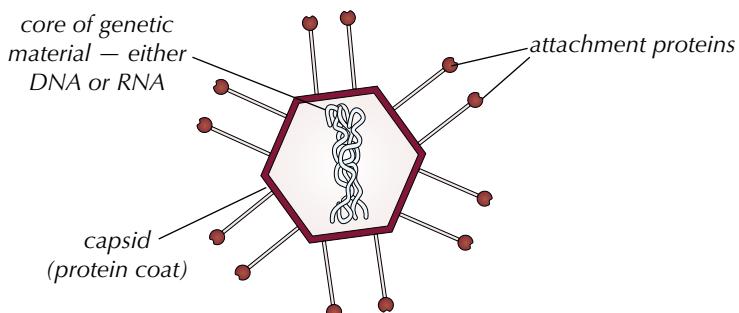


Figure 5: The general structure of a virus.

Viral replication

Because they're not alive, viruses don't undergo cell division. Instead, they inject their DNA or RNA into the host cell — this hijacked cell then uses its own 'machinery' (e.g. enzymes, ribosomes) to do the virus's dirty work and replicate the viral particles. The overall process is shown in Figure 6.

In order to inject their DNA or RNA, viruses first have to attach to the host cell surface. To do this they use their attachment proteins to bind to complementary receptor proteins on the cell-surface membrane of the host cells. Different viruses have different attachment proteins and therefore require different receptor proteins on host cells. As a result, some viruses can only infect one type of cell (e.g. some viruses can only infect one species of bacteria), while others can infect lots of different cells (e.g. influenza).

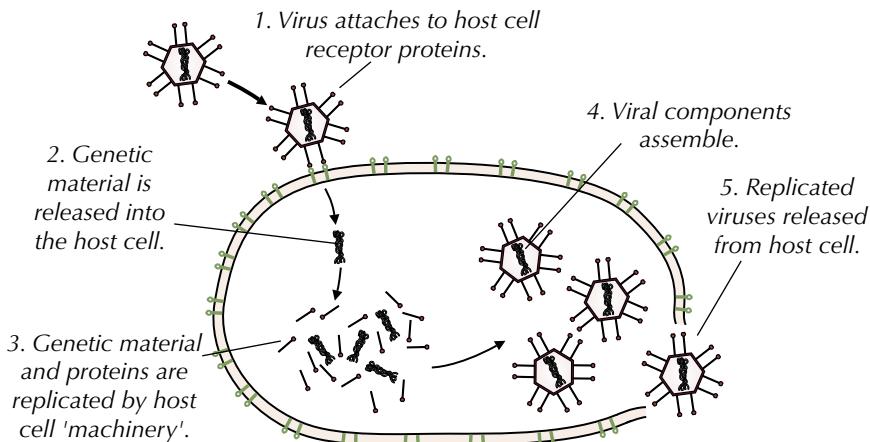


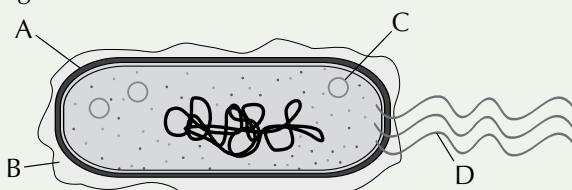
Figure 6: Example of viral replication by the host cell.

Tip: The receptor proteins on the host cells aren't just there to let viruses attach. They're actually proteins that play a role in the normal workings of the cell. Viruses have just evolved to exploit them.

Tip: Different viruses enter and leave the host cell in different ways and are replicated by the host cell in slightly different ways. E.g. HIV releases its capsid into the cell as well as its genetic material — see page 133. Figure 6 just shows the basic concept.

Practice Questions — Application

A scientist investigating the cause of an outbreak of food poisoning has found a type of bacterium in a faeces sample that he thinks is causing the illness. A diagram of the bacterium is shown below:



- 1 Name the features labelled A-D.
- 2 Suggest why features B and D make this bacterium well adapted to living in the gut.
- 3 Describe how the genetic material is arranged in this cell.

Practice Questions — Fact Recall

- 1 Where is murein used in prokaryotic cells?
- 2 Name and describe the process by which prokaryotic cells reproduce.
- 3 What is a capsid?
- 4 What is the role of viral attachment proteins?

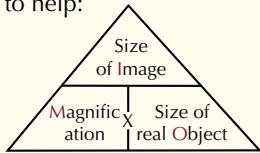
Learning Objectives:

- Be able to use this formula to calculate magnification:
$$\frac{\text{size of image}}{\text{size of real object}}$$
- Know the difference between magnification and resolution.
- Know the principles and limitations of optical microscopes and transmission and scanning electron microscopes.
- Appreciate that there was a considerable period of time during which the scientific community distinguished between artefacts and cell organelles.
- Understand how cell fractionation and ultracentrifugation are used to separate cell components.

Specification Reference 3.2.1.3

Exam Tip

If you find rearranging formulas hard you can use a formula triangle to help:



All you do is put your finger over the one you want and read off the formula. E.g. if you want the size of the real object, you put your finger over that and it leaves behind size of image ÷ magnification.

3. Analysis of Cell Components

Investigating cells, and what's in them, involves digging out your microscope.

Magnification and resolution of microscopes

We all know that microscopes produce a magnified image of a sample, but resolution is just as important...

Magnification

Magnification is how much bigger the image is than the specimen (the sample you're looking at). It's calculated using this formula:

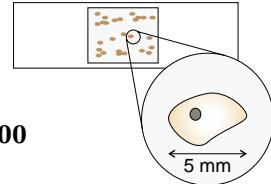
$$\text{magnification} = \frac{\text{size of image}}{\text{size of real object}}$$

Examples — Maths Skills

Calculating magnification

If you have a magnified image that's 5 mm wide and your specimen is 0.05 mm wide the magnification is:

$$\text{magnification} = \frac{\text{size of image}}{\text{size of real object}} = \frac{5}{0.05} = \times 100$$



Calculating size of image

If your specimen is 0.1 mm wide and the magnification of the microscope is $\times 20$, then the size of the image is:

$$\begin{aligned}\text{size of image} &= \text{magnification} \times \text{size of real object} \\ &= 20 \times 0.1 = 2 \text{ mm}\end{aligned}$$

Calculating size of real object

If you have a magnified image that's 5 mm wide and the magnification is $\times 50$, then the size of the real object (i.e. the size of the specimen you're looking at) is:

$$\text{size of real object} = \frac{\text{size of image}}{\text{magnification}} = \frac{5}{50} = 0.1 \text{ mm}$$

When you're calculating magnification you need to make sure that all lengths are in the same unit, e.g. all in millimetres. When dealing with microscopes these units can get pretty tiny. The table below shows common units:

| To convert | Unit | How many millimetres it is: | To convert |
|---------------|------------------------------|-----------------------------|-------------|
| $\times 1000$ | Millimetre (mm) | 1 mm | $\div 1000$ |
| $\times 1000$ | Micrometre (μm) | 0.001 mm | $\div 1000$ |
| | Nanometre (nm) | 0.000001 mm | |

The table shows that millimetres are three orders of magnitude (10^3 or 1000 times) bigger than micrometres, which are three orders of magnitude bigger than nanometres.

Example — Maths Skills

To convert from a smaller unit to a bigger unit you divide by 1000.

So to convert 6 micrometres to millimetres you divide 6 by 1000
 $= 0.006 \text{ mm}$. To go from a bigger unit to a smaller unit you times by 1000.

Resolution

Resolution is how detailed the image is. More specifically, it's how well a microscope distinguishes between two points that are close together. If a microscope lens can't separate two objects, then increasing the magnification won't help.

Tip: A microscope can't distinguish between objects that are smaller than its maximum resolution.

Example

When you look at a car in the dark that's a long way away you see the two headlights as one light. This is because your eyes can't distinguish between the two points at that distance — your eyes produce a low resolution image. When the car gets a bit closer you can see both headlights — a higher resolution image.

Practice Questions — Application

Q1 Image A shows a cartilage cell under a $\times 3150$ microscope.

- What is the diameter of the nucleus (labelled A) in millimetres?
- What is the diameter of the cell (labelled B) in millimetres?

Q2 A researcher is examining some ribosomes under a microscope.

Ribosomes are around 0.00002 mm long. How long will the image appear through a $\times 40$ microscope? Give your answer in standard form.

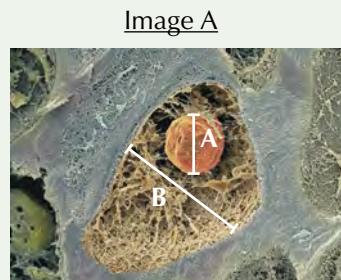


Image B

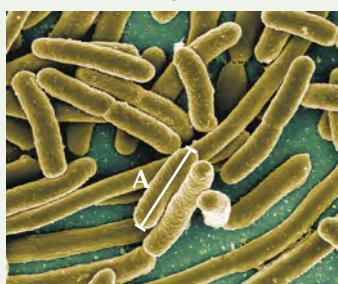


Image C



Q3 Image B shows some bacteria. It was taken using a $\times 7000$ microscope. How long is the bacterium labelled A, in micrometres?

Q4 Rhinovirus particles are around $0.023\text{ }\mu\text{m}$ in diameter. What will the diameter of the image be through a $\times 1500$ microscope? Give your answer in millimetres.

Q5 Image C shows a blood clot in an artery (labelled A). The clot is 2 mm in diameter.

- What is the magnification of the microscope?
- The diameter of the artery is 3 mm . If the same specimen was examined under a $\times 50$ microscope, what would the diameter of the artery in the image be?

Q6 A mitochondrion is $10\text{ }\mu\text{m}$ long. In a microscope image it is 10 mm . What is the magnification of the microscope?

Exam Tip

You might have to use standard form in your exam. It's when numbers are written to the power of 10, e.g. 2×10^{-5} instead of 0.00002 mm . See p. 7.

Exam Tip

Don't forget that the units need to be the same, e.g. all in millimetres, or all in micrometres.

Exam Tip

In the exam, you could be given a micrograph with a scale bar drawn on it. E.g. $\frac{1}{10}\text{ }\mu\text{m}$. If so, you can use this bar to work out the size of the real object. E.g. if the specimen is 4 bars long, it would measure: $4 \times 10 = 40\text{ }\mu\text{m}$.

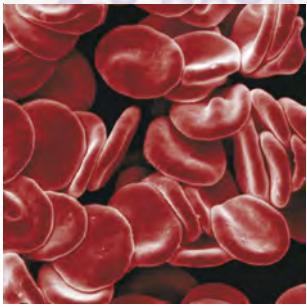
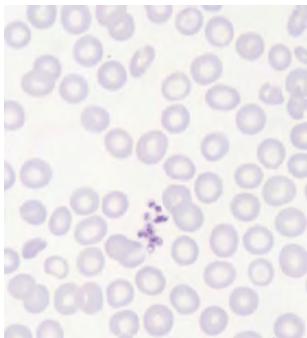


Figure 1: Red blood cells seen under an optical microscope (top) and an electron microscope (bottom).

Types of microscope

Optical (light) microscopes

They use light to form an image. They have a maximum resolution of about 0.2 micrometres (μm). This means you can't use an optical microscope to view organelles smaller than 0.2 μm . That includes ribosomes, the endoplasmic reticulum and lysosomes. You may be able to make out mitochondria — but not in perfect detail. You can also see the nucleus. The maximum useful magnification of an optical microscope is about $\times 1500$.

Electron microscopes

They use electrons to form an image. They have a higher resolution than optical microscopes, so give a more detailed image (and can be used to look at more organelles). They have a maximum resolution of about 0.0002 micrometres (μm). (About 1000 times higher than optical microscopes.) The maximum useful magnification of an electron microscope is about $\times 1\,500\,000$. Electron microscopes produce black and white images, but these are often coloured by a computer.

| | Optical microscope | Electron microscope |
|---------------|--|--|
| Magnification | Lower (maximum of $\times 1500$) | Higher (maximum of $\times 1\,500\,000$) |
| Resolution | Lower (maximum of 0.2 μm) | Higher (maximum of 0.0002 μm) |

Figure 2: Comparison table of optical and electron microscope features.

Types of electron microscope

Transmission electron microscopes (TEMs)

TEMs use electromagnets to focus a beam of electrons, which is then transmitted through the specimen. Denser parts of the specimen absorb more electrons, which makes them look darker on the image you end up with. TEMs are good because they give high resolution images, so you see the internal structure of organelles like chloroplasts. But you've got to view the specimen in a vacuum, so they're no good for looking at living organisms. They can also only be used on thin specimens.

Scanning electron microscopes (SEMs)

SEMs scan a beam of electrons across the specimen. This knocks off electrons from the specimen, which are gathered in a cathode ray tube to form an image. The images you end up with show the surface of the specimen and they can be 3-D. SEMs are good because they can be used on thick specimens, but they give lower resolution images than TEMs.

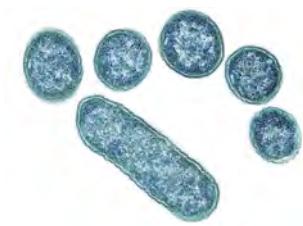


Figure 3: A TEM (top), and SEM (bottom) of *E.coli* bacteria.

| | TEMs | SEMs |
|---------------|--|--|
| Advantages | Give high resolution images, so shows small objects. | Can be used on thick specimens. Can be 3-D. |
| Disadvantages | Can only be used on thin specimens. Can only be used on non-living specimens. | Give lower resolution images than TEMs. Can only be used on non-living specimens. |

Figure 4: Comparison table of TEM and SEM features.

Preparing microscope slides

If you want to look at a specimen with an optical microscope, you'll need to put it on a microscope slide (strip of clear glass or plastic) first. This is often done using a **temporary mount** (also known as a wet mount). This is where the specimen is suspended in a drop of liquid (e.g. water, oil) on the slide.

1. Start by pipetting a small drop of water onto the centre of the slide.
2. Then use tweezers to place a thin section of your specimen on top of the water drop. (Your specimen needs to let light through it for you to be able to see it clearly under the microscope — so if you've got quite a thick specimen, you'll need to take a thin slice to use on your slide).
3. Add a drop of a stain. Stains are used to highlight objects in a cell.

Examples

- Eosin is used to make the cytoplasm show up.
- Iodine in potassium iodide solution (see page 28) is used to stain starch grains in plant cells.

4. Finally, add the cover slip (a square of clear glass or plastic that protects the specimen). To do so, stand the slip upright on the slide, next to the water droplet. Then carefully tilt and lower it so it covers the specimen. Try not to get any air bubbles under there (see below) — they'll obstruct your view of the specimen. These steps are shown in Figure 5.

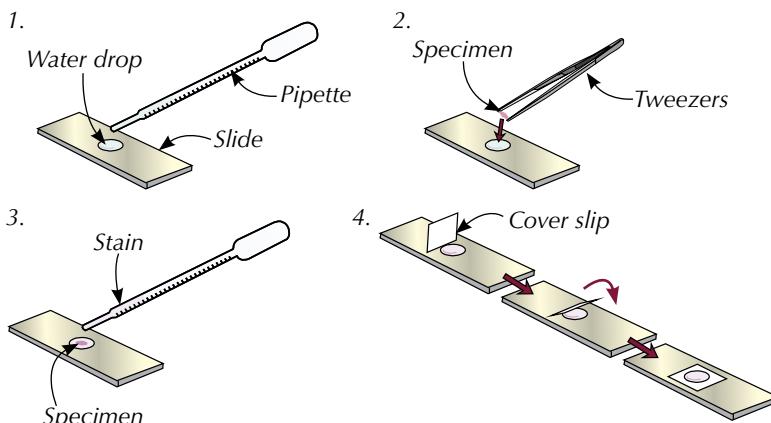


Figure 5: Preparation of a temporary mount microscope slide.

Tip: You can also get dry mounts, where the specimen is put on the slide without being suspended in liquid.

Tip: Make sure that you're aware of any hazards, particularly for any stains you're using, before you start preparing your slide.

Tip: Temporary mounts can't be stored for very long — that's why they're temporary. They're good for looking at organisms that live in water though, e.g. algae.



Figure 6: A scientist adding a drop of stain during the preparation of a temporary mount microscope slide.



Figure 7: A close up of a bee's eye, taken with an electron microscope, showing artefacts (purple and brown circles) caused by incorrect preparation of the specimen.

Microscope artefacts

Artefacts are things that you can see down the microscope that aren't part of the cell or specimen that you're looking at. They can be anything from bits of dust, air bubbles and fingerprints, to inaccuracies caused by squashing and staining your sample. Artefacts are usually made during the preparation of your specimen and shouldn't really be there at all.

Artefacts are especially common in electron micrographs because specimens need a lot of preparation before you can view them under an electron microscope.

The first scientists to use electron microscopes could only distinguish between artefacts and organelles by repeatedly preparing specimens in different ways. If an object could be seen with one preparation technique, but not another, it was more likely to be an artefact than an organelle.

Cell fractionation

Suppose you wanted to look at some organelles under an electron microscope. First you'd need to separate them from the rest of the cell — you can do this by cell fractionation. There are three steps to this technique:

1. Homogenisation — breaking up the cells

Homogenisation can be done in several different ways, e.g. by vibrating the cells or by grinding the cells up in a blender. This breaks up the plasma membrane and releases the organelles into solution.

The solution must be kept ice-cold, to reduce the activity of enzymes that break down organelles. The solution should also be isotonic — this means it should have the same concentration of chemicals as the cells being broken down, to prevent damage to the organelles through osmosis. A buffer solution should be added to maintain the pH.

Tip: There's loads more about osmosis on pages 106-107.

Tip: Filtration separates cell debris from the organelles, it doesn't separate out the different organelles (that's the job of ultracentrifugation).



Figure 8: A centrifuge.

Exam Tip

You can remember the order the organelles separate out in (Nuclei, Chloroplasts, Mitochondria, Lysosomes, ER, Ribosomes) using “Naughty Clever Monkeys Like Eating Red Raspberries”.

2. Filtration — getting rid of the big bits

Next, the homogenised cell solution is filtered through a gauze to separate any large cell debris or tissue debris, like connective tissue, from the organelles. The organelles are much smaller than the debris, so they pass through the gauze.

3. Ultracentrifugation — separating the organelles

After filtration, you're left with a solution containing a mixture of organelles. To separate a particular organelle from all the others you use ultracentrifugation:

- The cell fragments are poured into a tube. The tube is put into a centrifuge (a machine that separates material by spinning) and is spun at a low speed. The heaviest organelles, like nuclei, get flung to the bottom of the tube by the centrifuge. They form a thick sediment at the bottom — the pellet. The rest of the organelles stay suspended in the fluid above the sediment — the supernatant.
- The supernatant is drained off, poured into another tube, and spun in the centrifuge at a higher speed. Again, the heaviest organelles form a pellet at the bottom of the tube. The supernatant containing the rest of the organelles is drained off and spun in the centrifuge at an even higher speed.
- This process is repeated at higher and higher speeds, until all the organelles are separated out — see Figure 9. Each time, the pellet at the bottom of the tube is made up of lighter and lighter organelles.

The organelles are separated in order of mass (from heaviest to lightest) — this order is usually: nuclei, then mitochondria, then lysosomes, then endoplasmic reticulum, and finally ribosomes. In plant cells, the chloroplasts come out after the nuclei, but before the mitochondria.

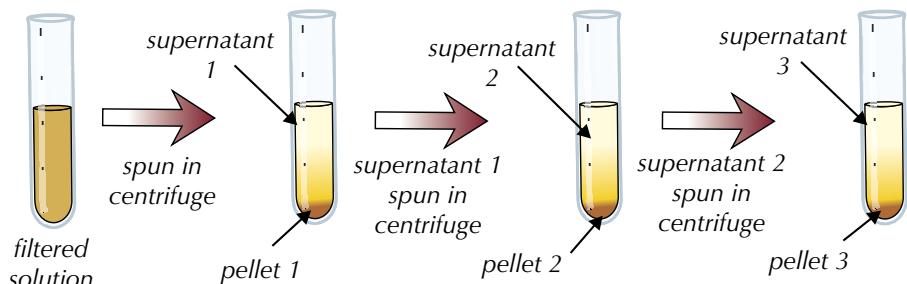


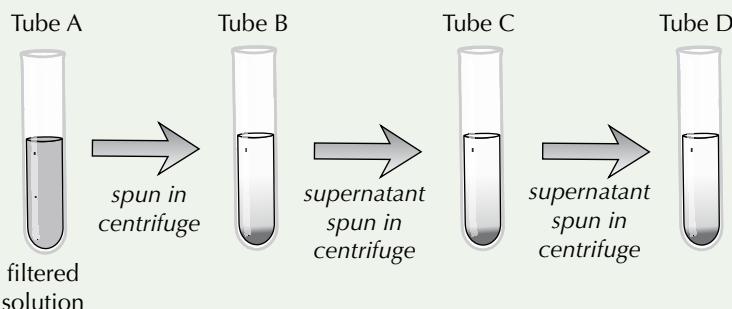
Figure 9: Ultracentrifugation.

Practice Questions — Application

Q1 Suggest what type of microscope you would use in each of the following scenarios. Give a reason for each answer.

- Studying how *E.coli* bacteria replicate.
- Studying the 3-D structure of red blood cells.
- Studying virus particles that are 0.1 µm in diameter.

Q2 The diagram below shows the first few steps in the ultracentrifugation of animal tissue.



Which organelles would you expect to find in:

- Tube A
- The pellet in Tube B
- The supernatant in Tube D

Tip: For Q1, you need to think about the advantages and disadvantages of each type of microscope.

Practice Questions — Fact Recall

Q1 What is the formula for calculating the magnification of a microscope?

Q2 What's the difference between the magnification of a microscope and its resolution?

Q3 What is the maximum resolution for:

- An optical microscope?
- An electron microscope?

Q4 Which has a higher maximum magnification, an optical microscope or an electron microscope?

Q5 What type of microscope would you use to study lysosomes?

Q6 How do transmission electron microscopes work?

Q7 How do scanning electron microscopes work?

Q8 Give one advantage and one disadvantage of TEMs.

Q9 Give one advantage of SEMs over TEMs.

Q10 What is a temporary mount microscope slide?

Q11 What is a microscope artefact?

Q12 How did the first scientists to use electron microscopes distinguish between artefacts and organelles?

Q13 Give two ways homogenisation for cell fractionation is done.

Q14 Describe what happens at the filtration step of cell fractionation and explain why it is carried out.

Learning Objectives:

- Know that within multicellular organisms, not all cells retain the ability to divide — cells that do show a cell cycle.
- Know that DNA replication occurs during interphase of the cell cycle.
- Know that mitosis is the part of the cell cycle in which a eukaryotic cell divides to produce two daughter cells, each with the identical copies of DNA produced by the parent cell during DNA replication.
- Recall the behaviour of chromosomes in interphase and at each stage of mitosis.
- Know the role of spindle fibres attached to centromeres in the separation of chromatids.
- Know that division of the cytoplasm (cytokinesis) usually occurs, producing two new cells.
- Be able to recognise the stages of the cell cycle.
- Be able to explain the appearance of cells at each stage of mitosis.
- Know that mitosis is a controlled process and that uncontrolled cell division can lead to the formation of tumours and of cancers.
- Know that many cancer treatments are directed at controlling the rate of cell division.

Specification Reference 3.2.2

4. Cell Division — Mitosis

We need new cells for growth and to replace damaged tissue, so our body cells need to be able to make more of themselves. They do this during the cell cycle, which includes mitosis.

The cell cycle

In multicellular organisms, not all cells keep their ability to divide. The ones that do follow a process called the cell cycle. The cell cycle starts when a cell has been produced by cell division and ends with the cell dividing to produce two identical cells. The cell cycle (see Figure 1) consists of a period of cell growth and DNA replication, called **interphase**, and a period of cell division, called **mitosis**. Interphase (cell growth) is subdivided into three separate growth stages. These are called G_1 , S and G_2 .

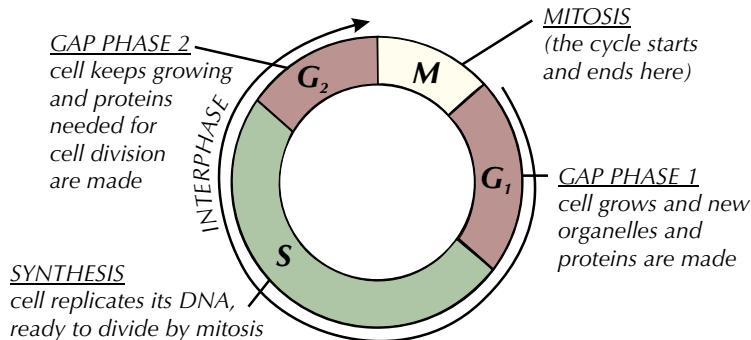
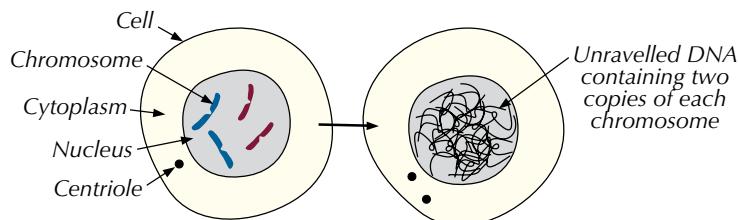


Figure 1: Stages of the cell cycle.

Interphase

During interphase the cell carries out normal functions, but also prepares to divide. The cell's DNA is unravelled and replicated, to double its genetic content. The organelles are also replicated so it has spare ones, and its ATP content is increased (ATP provides the energy needed for cell division).



Mitosis

There are two types of cell division — mitosis and meiosis (see p. 218 for more on meiosis). Mitosis is the form of cell division that occurs during the cell cycle. In mitosis, a parent cell divides to produce two genetically identical daughter cells (they contain an exact copy of the DNA of the parent cell). Mitosis is needed for the growth of multicellular organisms (like us) and for repairing damaged tissues. How else do you think you get from being a baby to being a big, strapping teenager — it's because the cells in our bodies grow and divide.

Mitosis is really one continuous process, but it's described as a series of division stages — prophase, metaphase, anaphase and telophase (see the next page).

The structure of chromosomes in mitosis

Before we go into the detail of mitosis, you need to know more about the structure of chromosomes. As mitosis begins, the chromosomes are made of two strands joined in the middle by a **centromere**. The separate strands are called **chromatids**. Two strands on the same chromosome are called **sister chromatids**. There are two strands because each chromosome has already made an identical copy of itself during interphase. When mitosis is over, the chromatids end up as one-strand chromosomes in the new daughter cells.

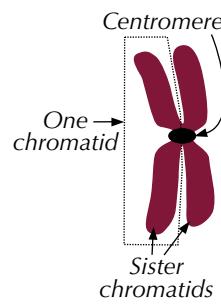


Figure 2: Interphase in bluebell cells.

1. Prophase

The chromosomes condense, getting shorter and fatter. Tiny bundles of protein called centrioles start moving to opposite ends of the cell, forming a network of protein fibres across it called the spindle. The nuclear envelope (the membrane around the nucleus) breaks down and chromosomes lie free in the cytoplasm.

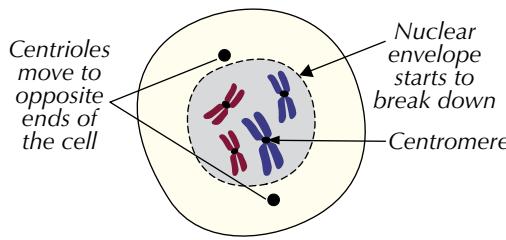


Figure 3: Prophase in bluebell cells.

2. Metaphase

The chromosomes (each with two chromatids) line up along the middle of the cell and become attached to the spindle by their centromere.

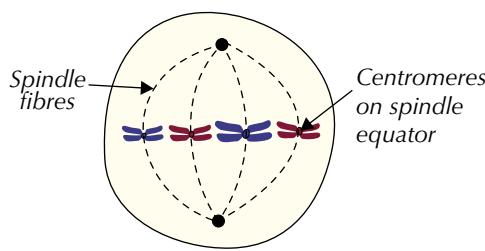


Figure 4: Metaphase in bluebell cells.

3. Anaphase

The centromeres divide, separating each pair of sister chromatids. The spindles contract, pulling chromatids to opposite poles (ends) of the spindle, centromere first. This makes the chromatids appear v-shaped.

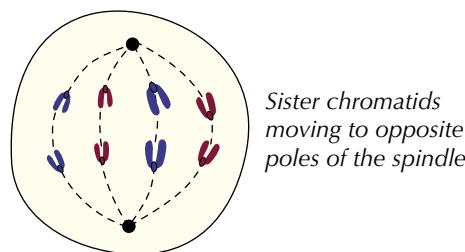


Figure 5: Anaphase in bluebell cells.

4. Telophase

The chromatids reach the opposite poles on the spindle. They uncoil and become long and thin again. They're now called chromosomes again. A nuclear envelope forms around each group of chromosomes, so there are now two nuclei. Division of the cytoplasm (**cytokinesis**, which starts in anaphase) finishes in telophase. There are now two daughter cells that are genetically identical to the original cell and to each other. Mitosis is finished and each daughter cell starts the interphase part of the cell cycle to get ready for the next round of mitosis.

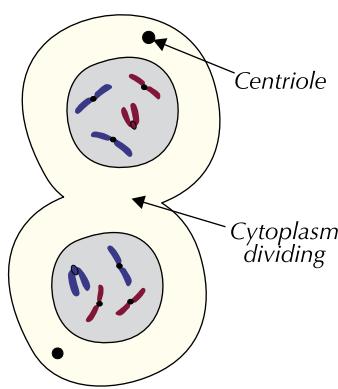


Figure 6: Telophase in bluebell cells.

Exam Tip

You could remember the order of the phases in mitosis (**p**rophase, **m**etaphase, **a**naphase, **t**elophase) by using, '**p**urple **m**ice are **t**asty'.

How long does each stage of mitosis take?

The time taken for each stage of mitosis varies depending on the cell type and the environmental conditions. You can calculate how long each stage of mitosis lasts if you're given the right information.

Example — Maths Skills

A scientist observes a section of growing tissue under the microscope. He counts 100 cells undergoing mitosis. Of those, 10 cells are in metaphase. One complete cell cycle of the tissue lasts 15 hours. How long do the cells spend in metaphase? Give your answer in minutes.

- The scientist has observed that 10 out of 100 cells are in metaphase. This suggests that the proportion of time the cells spend in metaphase must be 10/100th of the cell cycle.
- You're told that the cell cycle in these cells lasts 15 hours. That's $(15 \times 60 =)$ 900 minutes.
- So the cells spend: $\frac{10}{100} \times 900 = \textbf{90 minutes in metaphase.}$

Practice Questions — Application

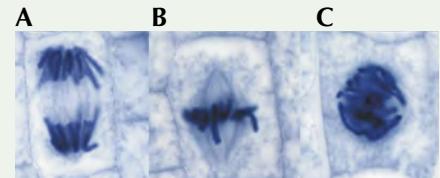
Exam Tip

You need to be able to recognise and explain the appearance of cells at each stage of mitosis for your exam.

Exam Tip

In graphs with two scales, make sure you match the correct line (or bar) to the correct scale before you read off a value.

- Q1 The photo on the right shows mitosis in onion cells.

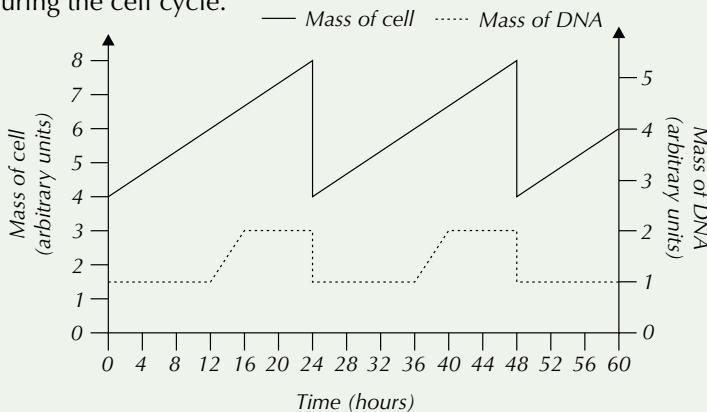


Which cell (A-C) is undergoing the following:

- metaphase,
- anaphase?

Give a reason for each of your answers.

- Q2 The graph below shows changes in the mass of a cell and its DNA during the cell cycle.



- During which hours does synthesis take place? Explain your answer.
- At which hours does mitosis take place? Explain your answer.
- i) How many cell divisions are shown on the graph?
ii) At what time will the next cell division take place?

- Q3 A scientist is looking at a tissue sample under a microscope. She counts 150 cells undergoing mitosis. Of those, 12 cells are in prophase. One complete cell cycle of the tissue lasts 0.70 days. How long do the cells spend in prophase? Give your answer in hours.

Cancer

Mitosis and the cell cycle are controlled by genes. Normally, when cells have divided enough times to make enough new cells, they stop. But if there's a mutation in a gene that controls cell division, the cells can grow out of control. The cells keep on dividing to make more and more cells, which form a tumour. Cancer is a tumour that invades surrounding tissue.

Tip: Mutations are changes in the base sequence of an organism's DNA (see page 223).

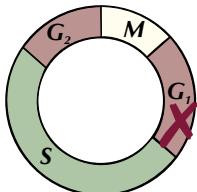
Cancer treatments

Some treatments for cancer are designed to control the rate of cell division in tumour cells by disrupting the cell cycle. This kills the tumour cells. These treatments don't distinguish tumour cells from normal cells though — they also kill normal body cells that are dividing. However, tumour cells divide much more frequently than normal cells, so the treatments are more likely to kill tumour cells.

Tip: Cancer is basically uncontrolled cell division.

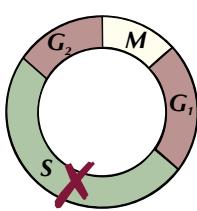
Examples

Some cell cycle targets of cancer treatments include:



G₁ (cell growth and protein production)

Some chemical drugs (chemotherapy) prevent the synthesis of enzymes needed for DNA replication. If these aren't produced, the cell is unable to enter the synthesis phase (S), disrupting the cell cycle and forcing the cell to kill itself.



S phase (DNA replication)

Radiation and some drugs damage DNA. At several points in the cell cycle, the DNA in the cell is checked for damage. If severe DNA damage is detected, the cell will kill itself — preventing further tumour growth.



Figure 7: Cancer of the knee — the tumour is sticking out of the leg.

Tip: Rapidly dividing cells, like hair cells and cells that line the gut, are often affected by cancer treatments. This can cause side effects like hair loss.

Practice Question — Application

- Q1 Methotrexate and vincristine are drugs used to treat cancer. Methotrexate blocks the formation of nucleotides within cells and vincristine prevents the formation of spindle fibres within the nuclei of cells. Which stage of the cell cycle is disrupted by:
a) methotrexate b) vincristine?

Tip: Don't let the tricky names of the drugs throw you when answering Q1 here. Just apply your knowledge to the information given in the question.

Practice Questions — Fact Recall

- Q1 What is the cell cycle?
Q2 Why is mitosis needed?
Q3 In what stage of the cell cycle does all the DNA unravel?
Q4 Describe what happens during prophase.
Q5 Describe what happens during telophase.
Q6 What is cytokinesis?
Q7 What is cancer?

Learning Objectives:

- Be able to prepare stained squashes of cells from plant root tips, and set up and use an optical microscope, to identify the stages of mitosis in the stained squashes and calculate the mitotic index (Required Practical 2).
- Know how to measure the size of an object viewed with an optical microscope.
- Be able to measure the apparent size of cells in the root tip and calculate their actual size using the formula:

$$\text{Actual size} = \frac{\text{size of image}}{\text{magnification}}$$

Specification Reference 3.2.2

Tip: Make sure you carry out a risk assessment (see p. 3) before you do this experiment, including assessing the specific risks for the particular staining technique you're using.

Tip: If you're using ethano-orcein as a stain, the tips will also need to be fixed in ethanoic acid before step 2.

Tip: Applying too much pressure to the slide or coverslip can break them. Be careful not to shatter the slide when cutting the root tip and be careful not to break the cover slip when squashing the cells.

5. Investigating Mitosis

You need to know how to carry out an experiment to investigate mitosis in cells — that includes preparing a root tip cell squash, using an optical microscope to view the cells and doing a few calculations based on what you see.

REQUIRED PRACTICAL 2

Preparing a root tip cell squash

You need to know how to prepare and stain a root tip in order to observe the stages of mitosis. Make sure you're wearing safety goggles and a lab coat before you start. You should also wear gloves when using stains.

- Add some 1 M hydrochloric acid to a boiling tube. There should be just enough acid in the tube to cover the root tip (see step 3) — so the acid should only be a few millimetres deep. Put the tube in a water bath that has been allowed to reach 60 °C.
- Use a scalpel to cut 1 cm from the tip from a growing root (e.g. of an onion). It needs to be the tip because that's where growth occurs and so that's where mitosis takes place.
- Carefully transfer the root tip into the boiling tube containing the acid. Incubate it for about 5 minutes.
- Use tweezers to remove the root tip from the tube and use a pipette to rinse it well with cold water. Leave the tip to dry on a paper towel.
- Place the root tip on a microscope slide (see Figure 1) and cut 2 mm from the very tip of it. Get rid of the rest.
- Use a mounted needle to break the tip open and spread the cells out thinly.
- Add a few drops of stain and leave it for a few minutes. The stain will make the chromosomes easier to see under a microscope. There are loads of different stains, all with crazy names — toluidine blue O, ethano-orcein, Feulgen stain. (If you're using the Feulgen stain, you'll need an extra rinse.)
- Place a cover slip over the cells and put a piece of folded filter paper on top. Push down firmly to squash the tissue. Squashing will make the tissue thinner and allow light to pass through it. Don't smear the cover slip sideways or you'll damage the chromosomes.
- Now you can look at all the stages of mitosis under an optical microscope (see next page). You should see something that looks like Figure 2.

Figure 1: Preparing a root tip squash slide.

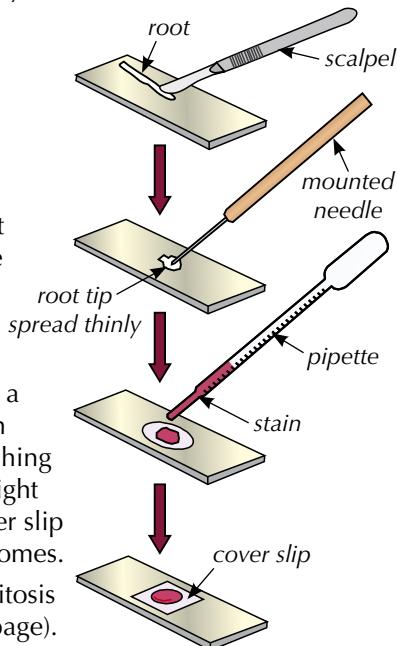
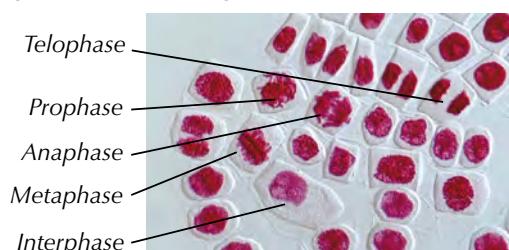


Figure 2: Optical microscope image of a stained root tip squash, showing cells in different stages of mitosis.



Using an optical microscope

REQUIRED
PRACTICAL 2

You need to know how to set up and use an optical microscope (see Figure 3) to observe your prepared root tip cells:

- Start by clipping the slide you've prepared onto the stage.
- Select the lowest-powered **objective lens** (i.e. the one that produces the lowest magnification).
- Use the coarse adjustment knob to bring the stage up to just below the objective lens.
- Look down the eyepiece (which contains the **ocular lens**). Use the coarse adjustment knob to move the stage downwards, away from the objective lens, until the image is roughly in focus.
- Adjust the focus with the fine adjustment knob until you get a clear image of what's on the slide.
- If you need to see the slide with greater magnification, swap to a higher-powered objective lens and refocus.

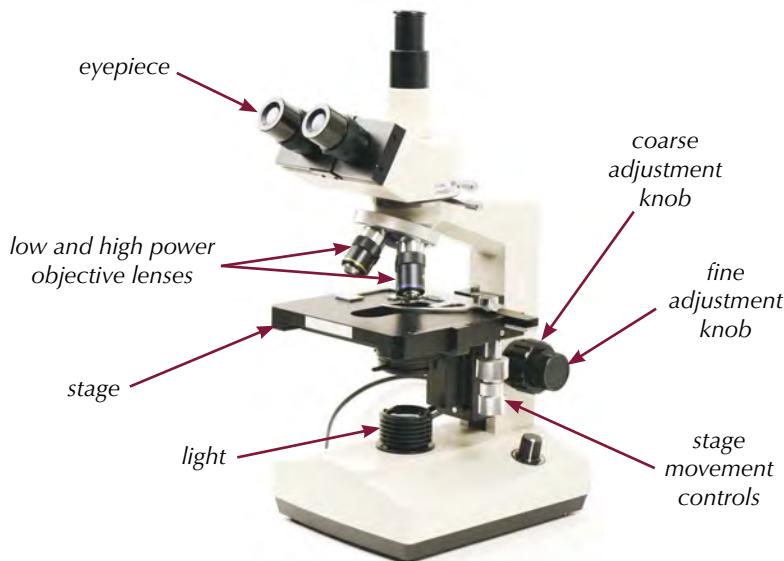


Figure 3: An optical microscope.

If you're asked to draw cells undergoing mitosis under the microscope, make sure the relative sizes of objects in your drawing are accurate and that you write down the magnification the specimen was viewed under. You'll also need to label your drawing and give it a title.

Calculating the mitotic index

REQUIRED
PRACTICAL 2

The mitotic index is the proportion of cells in a tissue sample that are undergoing mitosis. It lets you work out how quickly the tissue is growing and if there's anything weird going on. You can calculate the mitotic index of your squash cells using this formula:

$$\text{mitotic index} = \frac{\text{number of cells with visible chromosomes}}{\text{total number of cells observed}}$$

A plant root tip is constantly growing, so you'd expect a high mitotic index (i.e. lots of cells in mitosis). In other tissue samples, a high mitotic index could mean that tissue repair is taking place or that there is cancerous growth in the tissue.

Tip: The objective and ocular lenses magnify the specimen.

Tip: The **objective lenses** on your school microscope are likely to provide $\times 4$, $\times 10$ and $\times 40$ magnification. The **ocular lens** probably also provides $\times 10$ magnification. You need to multiply the magnification of the two lenses together to get the overall magnification. E.g. if you're using the $\times 40$ objective lens and the $\times 10$ ocular lens, the overall magnification will be $\times 10$ multiplied by $\times 40 = \times 400$.

Tip: Not all optical microscopes look the same, but they all have similar controls. You should be able to work out what does what on your school microscope from this diagram.

Tip: Your drawing should be done using a sharp pencil (not pen). Don't colour in or shade your drawing and make sure outlines are drawn neatly, not sketched.

Example — Maths Skills

Tip: Your answer should be between 0 and 1 (or between 0% and 100%). If it isn't, you know you've calculated the index wrong.

If you observed 30 cells, and 4 of them had visible chromosomes:

$$\text{mitotic index} = \frac{4}{30} = 0.13$$

The mitotic index can also be presented as a percentage.

All you have to do is multiply the figure by 100:

$$0.13 \times 100 = 13\%$$

Calculating the actual size of cells

You need to be able to calculate the size of the cells you're looking at. That's where the eyepiece graticule and stage micrometer come in — they're a bit like rulers.

An **eyepiece graticule** is fitted onto the eyepiece. It's like a transparent ruler with numbers, but no units. The **stage micrometer** is placed on the stage — it is a microscope slide with an accurate scale (it has units) and it's used to work out the value of the divisions on the eyepiece graticule at a particular magnification. This means that when you take the stage micrometer away and replace it with the slide containing your tissue sample, you'll be able to measure the size of the cells.

Example — Maths Skills

Tip: Remember: at a different magnification, one division on the stage micrometer will be equal to a different number of divisions on the eyepiece graticule — so the eyepiece graticule will need to be re-calibrated.

- Line up the eyepiece graticule and the stage micrometer (see Figure 4).
- Each division on the stage micrometer is 0.1 mm long.
- At this magnification, 1 division on the stage micrometer is the same as 4.5 divisions on the eyepiece graticule.
- To work out the size of 1 division on the eyepiece graticule, you need to divide 0.1 by 4.5: $0.1 \div 4.5 = 0.022\dots$ mm.

So if you look at a cell under the microscope at this magnification and it's 4 eyepiece divisions long (see Figure 5), you know it measures: $4 \times 0.022\dots = 0.088\dots = 0.09$ mm (1 s.f.)

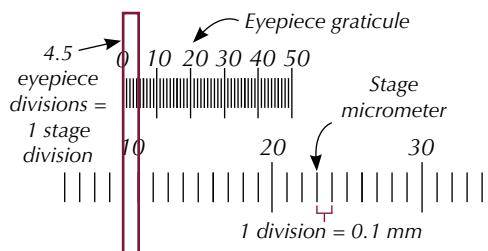


Figure 4: Lining up the eyepiece graticule and stage micrometer.

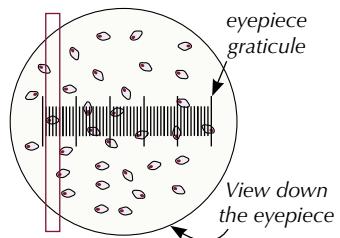


Figure 5: Using the calibrated eyepiece graticule to measure cells.

Tip: This is just a rearranged version of the magnification formula on page 80. It doesn't matter which one you use to do your calculations.

If you're given an image of cells under the microscope in the exam, you can calculate their actual size using this formula:

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

Example — Maths Skills

If the image of a cell measures 5 mm and the magnification is $\times 100$, then the actual size of the cell will be:

$$\text{actual size} = \frac{5}{100} = 0.05 \text{ mm}$$

Practice Questions — Application

- Q1 A student prepared a stained squash slide of cells from a hyacinth root tip, in order to investigate mitosis.
- Why was it necessary for the student to add a stain?
 - Name one suitable stain that he could have used.
 - Describe how the student should have squashed the tissue on the slide to avoid damaging the chromosomes.
 - Would you expect the mitotic index of these root tip cells to be higher or lower than the mitotic index of cells taken from a mature hyacinth leaf? Explain your answer.
- Q2 A microscope is set up with an eyepiece graticule and a stage micrometer. Each division on a stage micrometer is $10\text{ }\mu\text{m}$ long. At $\times 10$ magnification, 1 division of the stage micrometer is equal to 6.5 divisions on the eyepiece graticule.
- Calculate the size of 1 division on the eyepiece graticule. Give your answer to the nearest $0.1\text{ }\mu\text{m}$.
 - A specimen is viewed under this microscope at $\times 10$ magnification. It is 14 eyepiece divisions long. Use your answer to part a) to calculate the specimen's length. Give your answer to the nearest μm .
- Q3 A clinical scientist was analysing a tissue sample from a patient. He observed 750 cells and found that 207 of them had visible chromosomes.
- Calculate the mitotic index for this tissue sample. Give your answer as a percentage.
 - What does an abnormally high mitotic index suggest could be occurring in the tissue sample?
- The scientist also recorded the size of the cells.
- When using his optical microscope with a $\times 200$ magnification, one of the cells appeared to be 9.0 mm across. What is the actual size of this cell? Give your answer in millimetres.

Exam Tip

Be prepared for exam questions on the apparatus and techniques you've used in your Required Practicals.

Practice Questions — Fact Recall

- Q1 Describe how to focus an optical microscope on a specimen.
- Q2 What is an eyepiece graticule?
- Q3 What is the purpose of a stage micrometer?

Section Summary

Make sure you know:

- The structure, function and appearance of the following eukaryotic organelles: cell-surface membrane, nucleus (including the chromosomes and nucleolus), mitochondria, chloroplasts (plant and algal cells only), Golgi apparatus, Golgi vesicles, lysosomes, ribosomes, rough and smooth endoplasmic reticulum, cell wall (plant, algal and fungal cells) and cell vacuole (plant cells only).
- How in complex multicellular organisms, eukaryotic cells become specialised for a specific function.
- How to apply your knowledge of organelles to explain why different cells are suited to their function.

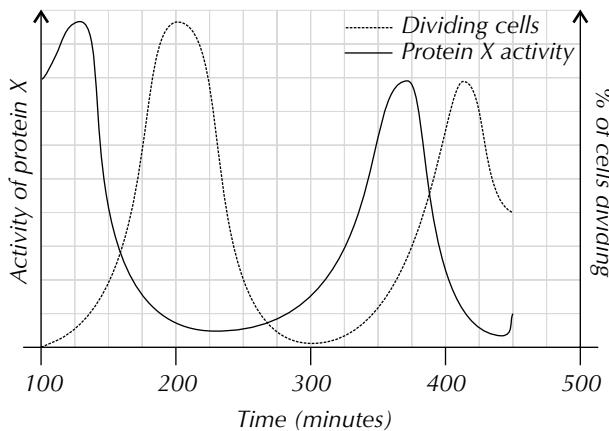
- That specialised cells are organised into tissues, tissues into organs and organs into systems.
- That prokaryotic cells are smaller and less complex than eukaryotic cells.
- How the structure of prokaryotic cells is different to eukaryotic cells — no membrane bound organelles in the cytoplasm (e.g. no nucleus), circular DNA and a murein cell wall.
- That some prokaryotic cells also have a capsule, flagella and plasmids.
- How a prokaryotic cell replicates by binary fission — the circular DNA and plasmids are replicated and then the cell divides to produce two daughter cells, each with a single copy of the circular DNA and a variable number of copies of plasmids.
- That viruses are non-living and acellular, so they don't undergo cell division. Instead, they invade host cells and use the host cell 'machinery' to replicate themselves.
- The structure of a typical virus, including the genetic material, capsid and attachment proteins.
- How to calculate magnification using the formula: magnification = size of image ÷ size of real object.
- The difference between magnification (how much bigger the image is than the sample) and resolution (how detailed the image is, based on the microscope's ability to distinguish between two points that are close together).
- The principles and limitations of optical microscopes, transmission electron microscopes (TEMs) and scanning electron microscopes (SEMs).
- How to prepare temporary mount microscope slides for viewing specimens with optical microscopes.
- That it wasn't always easy for early scientists using microscopes to distinguish between artefacts and cell organelles — and how they eventually did this.
- How cell fractionation separates out organelles — homogenisation, filtration and ultracentrifugation.
- That in multicellular organisms, not all cells are able to keep dividing.
- All about the eukaryotic cell cycle, including that DNA replication takes place in interphase and that the division of cells occurs during mitosis.
- That mitosis produces two daughter cells that are genetically identical to each other and to the parent cell.
- How the chromosomes behave during interphase, prophase, metaphase, anaphase and telophase of mitosis and the role of spindle fibres during mitosis.
- That mitosis ends with cytokinesis — division of the cytoplasm.
- How to recognise the stages of the cell cycle, including being able to explain the appearance of cells at each stage of mitosis.
- That when mitosis becomes uncontrolled, tumours and cancers can form.
- That cancer treatments are often aimed at controlling the rate of cell division.
- How to prepare stained squashes of root tip cells, use an optical microscope to observe the stages of mitosis in those cells, and calculate the mitotic index (the proportion of cells undergoing mitosis) of the cells (Required Practical 2).
- How to measure the size of an object viewed with an optical microscope, including how to use an eyepiece graticule and stage micrometer.
- How to use the formula $\text{actual size} = \text{size of image} \div \text{magnification}$, to calculate the actual size of an object viewed through a microscope.

Exam-style Questions

- 1 A scientist is studying secretory epithelial cells from the stomach under an optical microscope. The microscope has a magnification of $\times 100$ and a resolution of $0.2 \mu\text{m}$.
- 1.1 The ribosomes in the epithelial cells are 25 nm in diameter. Will the scientist be able to see them using the light microscope? Explain your answer. (1 mark)
- 1.2 The scientist sees an image of an epithelial cell that is 4 mm in diameter. Calculate the actual diameter of the cell. (1 mark)
- 1.3 One of the main functions of secretory epithelial cells in the stomach is to produce and secrete digestive enzymes. Suggest **one** organelle that is likely to be present in large numbers in the epithelial cells to aid this function. Explain your choice. (2 marks)
- 1.4 The scientist also separated the organelles by cell fractionation in order to study each one individually. Describe and explain the process of cell fractionation. (5 marks)
- 2 Penicillins are a group of antibiotics that are only effective against prokaryotic cells. They work by inhibiting cell wall synthesis, leading to cell lysis (bursting)
- 2.1 Explain why penicillin antibiotics can clear bacterial infections in humans without harming the infected individual's cells. (2 marks)
- 2.2 Antibiotics can be used to target other features of prokaryotic cells. Give an example of a feature that could be targeted and explain why it would be appropriate. (2 marks)
- 3 A team of scientists has been investigating the interphase stage of the cell cycle. The team analysed a sample of dividing cells using a machine called a flow cytometer.
- Figure 1** shows the amount of DNA against the number of cells with that amount of DNA.
- Figure 1 shows the amount of DNA against the number of cells with that amount of DNA.
- Figure 1
-
- Figure 1
- 3.1 Three phases of interphase are shown by the labels **A**, **B** and **C** on the graph. Name each phase and explain your answers. (3 marks)
- 3.2 Suggest why there are more cells in phase **A** than in phase **C**. (1 mark)

- 4 Proteins control all the different stages of the cell cycle. An experiment was conducted on the effect of protein X on mitosis in one species of yeast cells. At intervals the activity of the protein was measured and a microscope was used to determine the percentage of dividing yeast cells. The results are shown in **Figure 2**.

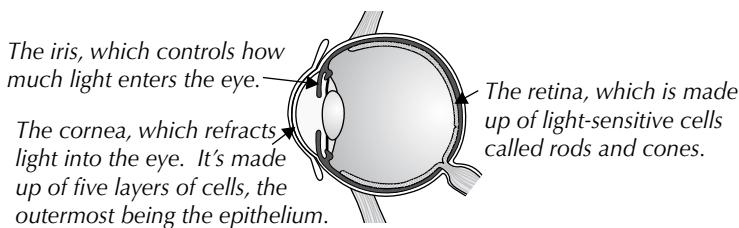
Figure 2



- 4.1 A scientist uses the data shown in the graph to conclude there is a causal relationship between protein X activity and cell division in all species of yeast. How far does the data support this conclusion? (3 marks)
- 4.2 Suggest a control that could have been carried out for this experiment and explain its purpose. (2 marks)

- 5 **Figure 3** shows an eye.

Figure 3



- 5.1 Is the eye a tissue or an organ?
Use evidence from **Figure 3** to explain your answer. (2 marks)
- 5.2 In normal cells there is a protein called Rb that stops the cell from leaving the G₁ phase of the cell cycle unless the cell needs to divide. A mutation in the gene for Rb can cause retinoblastoma — an eye cancer. Explain why. (3 marks)

1. Cell Membranes — The Basics

Cell membranes are the boundaries of cells, but there's an awful lot more to them than that...

Membrane function

All cells are surrounded by membranes. In eukaryotic cells, many of the organelles are surrounded by membranes too.

Cell-surface membranes

Cell-surface membranes surround cells. They are a barrier between the cell and its environment, controlling which substances enter and leave the cell. They're **partially permeable** — they let some molecules through but not others. Substances can move across the cell-surface membrane by **diffusion**, **osmosis** or **active transport** (see pages 102-112). The cell-surface membrane is sometimes called the plasma membrane.

Membranes within cells

The membranes around organelles divide the cell into different compartments — they act as a barrier between the organelle and the cytoplasm.

Example

The substances needed for respiration (like enzymes) are kept together inside a mitochondrion by the membrane surrounding the mitochondrion.

They are also partially permeable and control what substances enter and leave the organelle.

Example

RNA (see page 53) leaves the nucleus via the nuclear membrane (also called the nuclear envelope). DNA is too large to pass through the partially permeable membrane, so it remains in the nucleus.

Membrane structure

The basic structure of all cell membranes is pretty much the same. They're composed of lipids (mainly phospholipids — see page 30), proteins and carbohydrates (attached to proteins or lipids).

In 1972, the **fluid mosaic model** was suggested to describe the arrangement of molecules in the membrane — see Figure 2 (next page). In the model, phospholipid molecules form a continuous, double layer (called a bilayer). This bilayer is 'fluid' because the phospholipids are constantly moving.

Proteins are scattered through the bilayer, like tiles in a mosaic. These include **channel proteins** and **carrier proteins**, which allow large molecules and ions to pass through the membrane. Receptor proteins on the cell-surface membrane allow the cell to detect chemicals released from other cells.

The chemicals signal to the cell to respond in some way, e.g. the hormone insulin binds to receptor proteins on liver cells, which tells the cells to absorb glucose.

Learning Objectives:

- Know that the basic structure of all cell membranes, including cell-surface membranes and the membranes around the cell organelles of eukaryotes, is the same.
- Know the arrangement and any movement of phospholipids, proteins, glycoproteins and glycolipids in the fluid mosaic model of membrane structure.
- Understand how the nature of the phospholipid bilayer limits the diffusion of particles across cell membranes.
- Understand that cholesterol may also be present in cell membranes where it restricts the movement of other molecules making up the membrane.
- Know how to investigate the effect of a named variable on the permeability of cell-surface membranes (Required Practical 4).

Specification Reference 3.2.3

Tip: The phospholipid bilayer is about 7 nm thick.

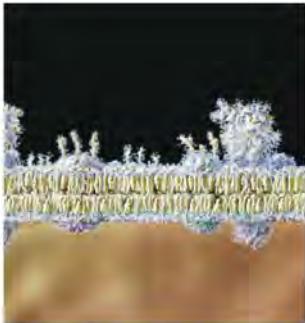


Figure 1: A computer model of the fluid mosaic model.

Some proteins are able to move sideways through the bilayer, while others are fixed in position. Some proteins have a carbohydrate attached — these are called **glycoproteins**. Some lipids also have a carbohydrate attached — these are called **glycolipids**. **Cholesterol** molecules are also present within the bilayer.

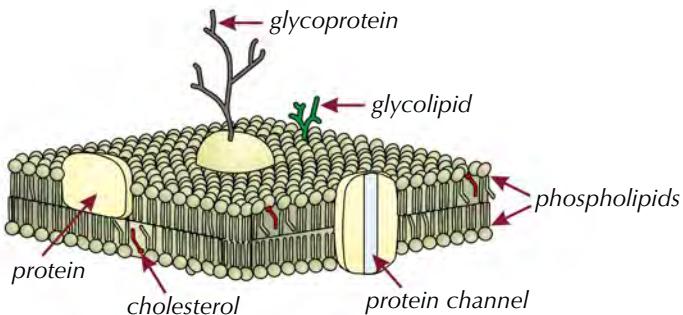


Figure 2: The fluid mosaic model of a cell membrane.

Membrane components

You need to know the roles of these two membrane components:

Tip: A polar molecule has one end with a slightly positive charge and one end with a slightly negative charge — see page 63. These charges are nowhere near as strong as the positive or negative charge on an ion, but they do help polar molecules to dissolve in water. Non-polar substances have no charges.

Tip: Water is actually a polar molecule, but it can diffuse (by osmosis) through the cell membrane because it's so small (see page 106).

Tip: There's more on phospholipids on pages 30-31.

Phospholipids

Phospholipid molecules form a barrier to dissolved (water-soluble) substances. Phospholipids have a 'head' and a 'tail'. The head is **hydrophilic** — it attracts water. The tail is **hydrophobic** — it repels water. The molecules automatically arrange themselves into a bilayer — the heads face out towards the water on either side of the membrane (see Figure 3).

The centre of the bilayer is hydrophobic so the membrane doesn't allow water-soluble substances (like ions and polar molecules) to diffuse through it. Small, non-polar substances (e.g. carbon dioxide) and water can diffuse through the membrane (see page 102).

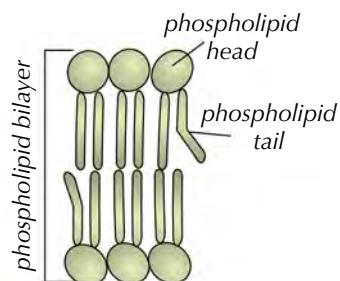


Figure 3: Phospholipid bilayer.

Cholesterol

Cholesterol gives the membrane stability. It is a type of lipid that's present in all cell membranes (except bacterial cell membranes). Cholesterol fits between the phospholipids (see Figure 4).

Cholesterol binds to the hydrophobic tails of the phospholipids, causing them to pack more closely together. This restricts the movement of the phospholipids, making the membrane less fluid and more rigid. Cholesterol helps to maintain the shape of animal cells (which don't have cell walls). This is particularly important for cells that aren't supported by other cells, e.g. red blood cells, which float free in the blood.



Figure 4: Cholesterol in the membrane.

Cholesterol also has hydrophobic regions, so it's able to create a further barrier to polar substances moving through the membrane.

Temperature and membranes

Cell membranes are affected by temperature — it affects how much the phospholipids in the bilayer can move, which affects membrane structure and permeability.

Temperatures below 0 °C

The phospholipids don't have much energy, so they can't move very much. They're packed closely together and the membrane is rigid. But channel proteins and carrier proteins in the membrane denature (lose structure and function), increasing the permeability of the membrane (see Point 1, Figure 5). Ice crystals may form and pierce the membrane, making it highly permeable when it thaws.

Temperatures between 0 and 45 °C

The phospholipids can move around and aren't packed as tightly together — the membrane is partially permeable (see Point 2, Figure 5). As the temperature increases the phospholipids move more because they have more energy — this increases the permeability of the membrane.

Temperatures above 45 °C

The phospholipid bilayer starts to melt (break down) and the membrane becomes more permeable. Water inside the cell expands, putting pressure on the membrane. Channel proteins and carrier proteins in the membrane denature so they can't control what enters or leaves the cell — this increases the permeability of the membrane (Point 3, Figure 5).

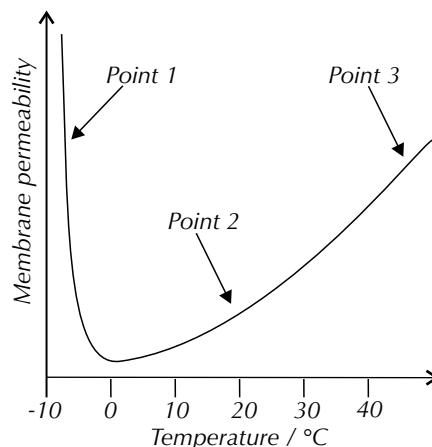


Figure 5: Graph to show the effect of temperature on membrane permeability.

Tip: You may remember from Topic 1A that proteins (e.g. enzymes) denature at high temperatures. Well, very cold temperatures (i.e. those below 0 °C) can cause proteins to denature too.

Practice Questions — Application

- Q1 Suggest a function of each of the following membranes:
 - a) the membrane surrounding a chloroplast.
 - b) the membrane surrounding a bacterial cell.
- Q2 Chloride ions (Cl^-) need to pass through the cell-surface membrane to get inside the cell. How might they move across the membrane?
- Q3 The protein content of a typical cell membrane is around 50%. In energy-releasing organelles, such as mitochondria, the amount rises to around 75%. Suggest a reason for this difference.
- Q4 A person removes some raspberries from the freezer that have frozen solid and leaves them on a plate to defrost. When he returns, there's a red puddle on the plate around the fruit. Use your knowledge of cell membranes to explain what has happened.

Tip: Think about what happens when you cook fruit or vegetables — as you apply heat, the food softens and liquid is released. This is partly because the cell membranes start to break down and become more permeable.

Tip: You should assess all safety risks before proceeding with this experiment. Be especially careful when using a scalpel — make sure you cut away from yourself and that the blade is clean and sharp.

Tip: Place the tubes into the water bath gently to avoid splashing hot water on yourself. Use tongs to remove them after the experiment — they may be hot.

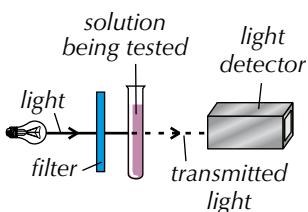


Figure 6: A diagram showing how a colorimeter works.

Tip: When you're handling cuvettes you need to wipe away any marks or moisture from the sides the light will be passing through. You should also gently tap the cuvette to remove any air bubbles.

Tip: Your teacher will show you how to calibrate the colorimeter you are using to zero.



Figure 7: A cuvette being placed inside a colorimeter.

Investigating cell membrane permeability

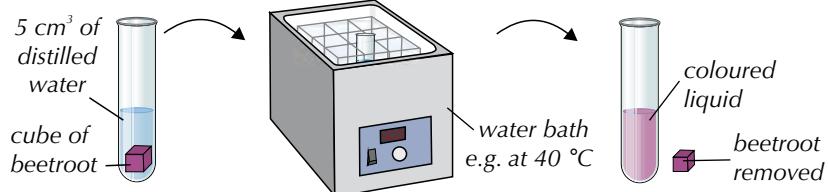
You can investigate how different variables (e.g. temperature and solvent concentration) affect cell membrane permeability by doing experiments using beetroot. Beetroot cells contain a coloured pigment that leaks out — the higher the permeability of the membrane, the more pigment leaks out of the cell.

REQUIRED PRACTICAL 4

Example — Investigating temperature

Here's how you could investigate how temperature affects beetroot membrane permeability:

1. Use a scalpel to carefully cut five equal sized pieces of beetroot. (Make sure you do your cutting on a cutting board.) Rinse the pieces to remove any pigment released during cutting.
2. Add the five pieces to five different test tubes, each containing 5 cm³ of water. Use a measuring cylinder or pipette to measure the water.
3. Place each test tube in a water bath at a different temperature, e.g. 10 °C, 20 °C, 30 °C, 40 °C, 50 °C, for the same length of time (measured using a stopwatch).
4. Remove the pieces of beetroot from the tubes, leaving just the coloured liquid.



5. Now you need to use a **colorimeter** — a machine that passes light of a specific wavelength through a liquid and measures how much of that light is absorbed. Many colorimeters use filters to make sure the light passing through the liquid is at the desired wavelength.
6. Firstly, switch the colorimeter on and allow five minutes for it to stabilise. Then set up the colorimeter so you're using a blue filter (or a wavelength of about 470 nm).
7. Add distilled water to a cuvette so it is three quarters full (a cuvette is a small container that fits inside a colorimeter — see Figure 7). Put the cuvette into the colorimeter. Two of the cuvette's sides may be ridged or frosted — you need to make sure you put the cuvette into the colorimeter the correct way, so that the light will be passing through the clear sides. Calibrate the machine to zero.
8. Next, use a pipette to transfer a sample of the liquid from the first test tube to a clean cuvette — again it should be about three quarters full.
9. Put the cuvette in the colorimeter and read and record the absorbance of the solution.
10. Repeat steps 8-9 for the liquids in the remaining four test tubes (using a clean pipette and cuvette each time).
11. You're now ready to analyse your results — bear in mind, the higher the absorbance reading, the more pigment released, so the higher the permeability of the membrane.

Depending on the resources you have available, you may be able to connect the colorimeter to a computer and use software to collect the data and draw a graph of the results.

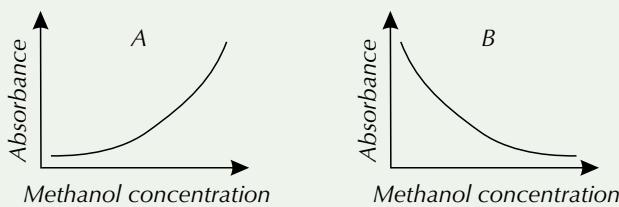
Investigating the effect of solvents

You could do a similar experiment with beetroot to investigate the effect of solvents on the permeability of cell membranes, i.e. by placing the beetroot cubes in different concentrations of a particular solvent (such as alcohol or acetone). Surrounding cells in an increasing concentration of a solvent increases membrane permeability because the solvent dissolves the lipids in the cell membrane, causing it to lose its structure.

Tip: If you're investigating the effect of solvents, remember to keep all other variables the same. Also, make sure you wear eye protection when working with solvents and that there are no naked flames around.

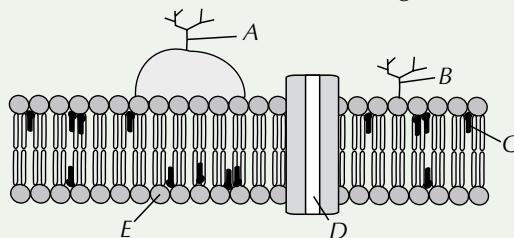
Practice Question — Application

- Q1 An experiment was carried out to investigate the effect of increasing methanol concentration on the permeability of beetroot cell membranes. Beetroot cubes were soaked in varying concentrations of methanol for a set amount of time, then a colorimeter was used to read the absorbance of the liquid once the beetroot cubes had been removed. The results of the experiment were used to produce a graph.
- Give four variables that should be controlled in this experiment.
 - Give two things that should be done with the colorimeter before it is used to measure the absorbance of the liquid samples.
 - Suggest which of the graphs below (A or B) was produced using the results of the experiment. Explain your answer.



Practice Questions — Fact Recall

- Q1 Identify the structures labelled A-E in the diagram below.



- Q2 Why is the phospholipid bilayer described as fluid?
Q3 Describe the movement of proteins within the bilayer.
Q4 What is a glycoprotein?
Q5 Explain the meaning of the terms 'hydrophilic' and hydrophobic'.
Q6 Explain why a cell membrane is an effective barrier against water-soluble substances.
Q7 How does the cell-surface membrane control what enters and leaves the cell?
Q8 Describe the role of cholesterol in a cell membrane.
Q9 Briefly describe how you could investigate the effect of temperature on the permeability of cell membranes.

Exam Tip

Not all diagrams of the fluid mosaic model look the same, so don't just memorise the pictures — make sure you learn what all the different components actually are and how they fit together.

Learning Objectives:

- Know that movement across cell membranes can occur by simple diffusion.
- Be able to explain how differences in gradients of concentration affect the rate of movement across cell membranes.
- Know that cells may be adapted for rapid transport across their internal or external membranes by an increase in surface area of their membranes, and be able to explain how this adaptation of specialised cells affects the rate of transport.
- Know that movement across cell membranes can also occur by facilitated diffusion (including the roles of carrier and channel proteins).
- Know that cells may be adapted for rapid transport across their internal or external membranes by an increase in the number of channel proteins and carrier proteins in their membranes, and be able to explain how this adaptation of specialised cells affects the rate of transport.

Specification Reference 3.2.3

Tip: Internal cell membranes are ones surrounding organelles, e.g. the mitochondria.

2. Diffusion

There are many ways substances move in and out of cells across the membrane. First up we have simple and facilitated diffusion...

The process of diffusion

Diffusion is the net movement of particles (molecules or ions) from an area of higher concentration to an area of lower concentration. Molecules will diffuse both ways, but the **net movement** will be to the area of lower concentration. This continues until particles are evenly distributed throughout the liquid or gas. The **concentration gradient** is the path from an area of higher concentration to an area of lower concentration. Particles diffuse down a concentration gradient.

Diffusion is a passive process — no energy is needed for it to happen. Particles can diffuse across cell membranes, as long as they can move freely through the membrane. When molecules diffuse directly through a cell membrane, it's also known as **simple diffusion**.

Example

Oxygen and carbon dioxide can diffuse easily through cell membranes because they're small, so they can pass through spaces between the phospholipids. They're also non-polar, which makes them soluble in lipids, so they can dissolve in the hydrophobic bilayer.

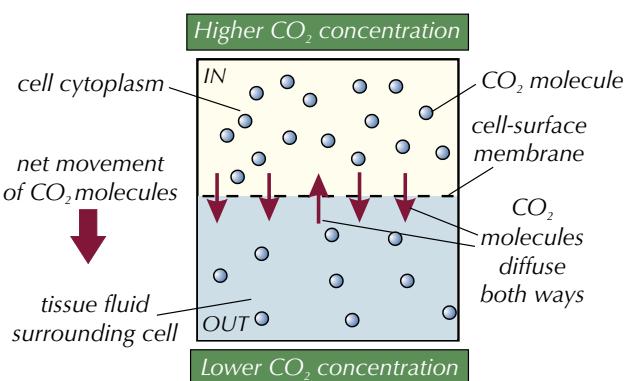


Figure 1: Diffusion of carbon dioxide across the cell-surface membrane.

Factors affecting the rate of diffusion

The rate of diffusion across both external and internal cell membranes can vary. Some specialised cells are adapted for rapid transport across their membranes. The rate of diffusion depends on:

- The concentration gradient — the higher it is, the faster the rate of diffusion. As diffusion takes place, the difference in concentration between the two sides of the membrane decreases until it reaches an equilibrium (i.e. the concentration on both sides is equal). This means that diffusion slows down over time.
- The thickness of the exchange surface — the thinner the exchange surface (i.e. the shorter the distance the particles have to travel), the faster the rate of diffusion.
- The surface area — the larger the surface area (e.g. of the cell-surface membrane), the faster the rate of diffusion.

Example

Some specialised cells (e.g. epithelial cells in the small intestine) have microvilli — projections formed by the cell-surface membrane folding up on itself (see p. 75). Microvilli give the cell a larger surface area — in human cells microvilli can increase the surface area by about 600 times. A larger surface area means that more particles can be exchanged in the same amount of time — increasing the rate of diffusion.

Facilitated diffusion

Some larger molecules (e.g. amino acids, glucose) would diffuse extremely slowly through the phospholipid bilayer because they're so big. Charged particles, e.g. ions and polar molecules, would also diffuse slowly — that's because they're water soluble, and the centre of the bilayer is hydrophobic (see page 98). So to speed things up, large or charged particles diffuse through **carrier proteins** or **channel proteins** in the cell membrane instead — this is called facilitated diffusion.

Like diffusion, facilitated diffusion moves particles down a concentration gradient, from a higher to a lower concentration. It's also a passive process — it doesn't use energy. There are two types of protein involved — carrier proteins and channel proteins.

Carrier proteins

Carrier proteins move large molecules across the membrane, down their concentration gradient. Different carrier proteins facilitate the diffusion of different molecules. Here's how they work:

- First, a large molecule attaches to a carrier protein in the membrane.
- Then, the protein changes shape.
- This releases the molecule on the opposite side of the membrane — see Figure 2.

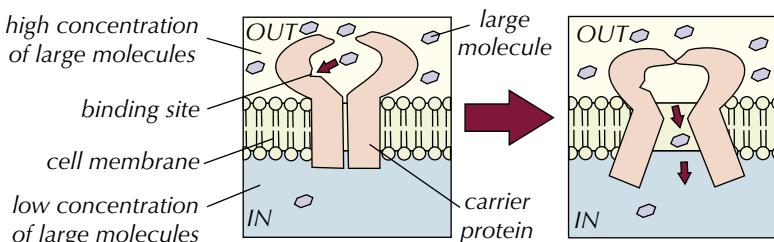


Figure 2: Movement of a molecule by carrier proteins.

Channel proteins

Channel proteins form pores in the membrane for charged particles to diffuse through (down their concentration gradient). Different channel proteins facilitate the diffusion of different charged particles.

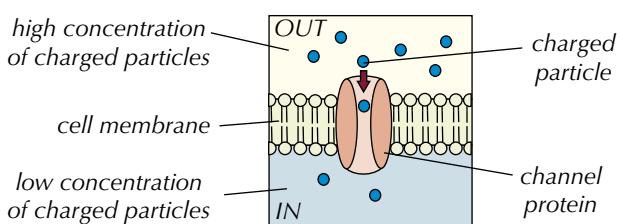


Figure 3: Movement of a charged particle by channel proteins.

Tip: Remember — small, non-polar substances and water can diffuse directly through the membrane.

Exam Tip

Always say down the concentration gradient in the exam, not across or along — or you won't get the marks.

Tip: Carrier proteins and channel proteins are called transport proteins.

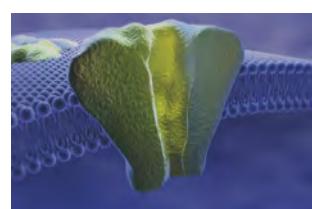


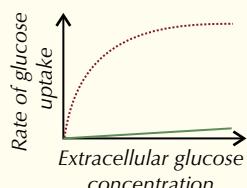
Figure 4: Computer model showing a cross section of a channel protein in the phospholipid bilayer.

Factors affecting the rate of facilitated diffusion

The rate of facilitated diffusion depends on:

- The **concentration gradient** — the higher the concentration gradient, the faster the rate of facilitated diffusion (up to a point, see below). As equilibrium is reached, the rate of facilitated diffusion will level off.
- The number of **channel** or **carrier proteins** — once all the proteins in a membrane are in use, facilitated diffusion can't happen any faster, even if you increase the concentration gradient.

Tip: The green line on this graph shows the rate of uptake if glucose was absorbed by simple diffusion — it's much slower than facilitated diffusion.



Tip: About 180 litres of water need re-absorbing every day.

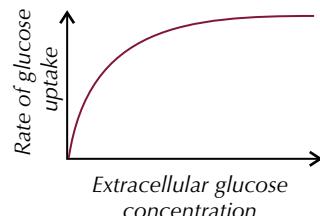
Tip: There's more on calculating rates from a graph on pages 12-14.

Tip: To find the units, divide the units on the y-axis by the units on the x-axis, just as you find the gradient.

Tip: Ms^{-1} means the same as M/s or moles per second.

Example

Glucose is absorbed from blood plasma into red blood cells via facilitated diffusion, using GLUT 1 carrier proteins. As the red line on the graph shows, the rate of uptake increases as the extracellular glucose concentration increases. The rate of uptake levels off as equilibrium is reached. After this, the rate of facilitated diffusion increases only slightly even at much greater glucose concentrations, as many of the GLUT 1 proteins are already in use.



So the greater the number of channel or carrier proteins in the cell membrane, the faster the rate of facilitated diffusion.

Example

Aquaporins are special channel proteins that allow the facilitated diffusion of water through cell membranes. Some kidney cells are adapted to have lots of aquaporins. The aquaporins allow the cells to reabsorb a lot of the water that would otherwise be excreted by the body.

Calculating the rate of diffusion

In the exams, you might be asked to calculate the rate of diffusion (or any other form of transport across a membrane) from a graph. For a straight line graph, this means finding the **gradient** of the line. For a curved graph, it means drawing a **tangent** and finding the gradient of the tangent.

Example — Maths Skills

The graph below shows the concentration of a particle in a cell over time. The concentration is decreasing as the particle diffuses out of the cell. Find the rate of diffusion at 3 seconds.

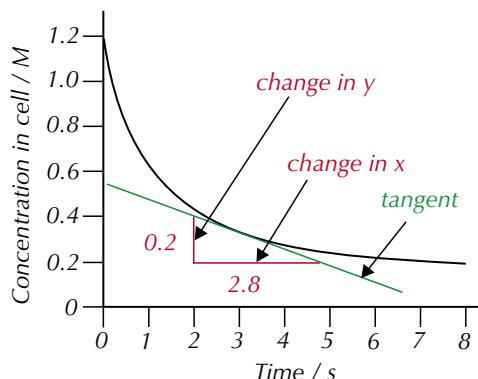
Step 1: Draw a tangent to the curve at 3 s.

Step 2: Calculate the gradient of the tangent:

$$\text{gradient} = \frac{\text{change in } y}{\text{change in } x} = \frac{0.2}{2.8} = 0.07$$

Step 3: Find the units to give the rate of diffusion:

$$\text{rate of diffusion} = 0.07 \text{ Ms}^{-1}$$



Practice Questions — Application

Q1 The photograph on the right shows ink diffusing through a beaker of water. Explain what is happening to the ink molecules.

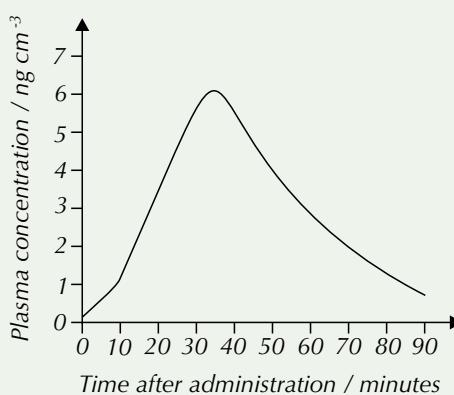


Q2 Carbon dioxide is a waste product of respiration and must be removed from cells. How will each of the following affect the rate of diffusion of carbon dioxide across a cell-surface membrane? Explain your answer in each case.

- Increasing the thickness of the cell membrane.
- Increasing the number of folds in the cell membrane.
- Reducing the concentration of carbon dioxide outside of the cell.

Q3 Simple diffusion and facilitated diffusion both move particles down their concentration gradient across a cell membrane. Suggest how you could determine whether a particular particle is being transported by simple or facilitated diffusion in an experimental setting. Explain your answer.

Q4 Following oral administration, a particular drug diffuses across the cell-surface membranes of cells in the digestive tract into the blood plasma. The graph on the right shows how the concentration of the drug in the blood plasma changes over time. Calculate the rate of diffusion 10–30 minutes after taking the drug.



Practice Questions — Fact Recall

- What is diffusion?
- Is simple diffusion an active or passive process?
- Give three factors that affect the rate of simple diffusion.
- Is facilitated diffusion an active or passive process?
- Briefly describe how a carrier protein transports molecules across a cell membrane.
- What is a channel protein?
- Describe the role of channel proteins in the transport of particles across a cell membrane.
- Explain how increasing the number of carrier and channel proteins in a membrane would affect the rate of facilitated diffusion.

Exam Tip

Make sure you don't get the roles of carrier and channel proteins mixed up in the exam — you could be throwing away easy marks.

Learning Objectives:

- Know that transport across the cell membrane can occur by osmosis.
- Be able to describe the process of osmosis in terms of water potential.
- Be able to explain how differences in water potential and a cell membrane's surface area affect the rate of movement across cell membranes.
- Know how to produce a dilution series of a solute and use it to produce a calibration curve with which to identify the water potential of plant tissue (Required Practical 3).

Specification Reference 3.2.3

Tip: Another way of looking at it is that pure water has the highest water potential and all solutions have a lower water potential than pure water.

Exam Tip

You should always use the term water potential in the exam — never say water concentration.

3. Osmosis

Osmosis is a special case of diffusion for water molecules...

What is osmosis?

Osmosis is the diffusion of water molecules across a partially permeable membrane, from an area of higher water potential (i.e. higher concentration of water molecules) to an area of lower water potential (i.e. lower concentration of water molecules). **Water potential** is the potential (likelihood) of water molecules to diffuse out of or into a solution.

Water molecules are small and can diffuse easily through the cell membrane, but large solute molecules can't.

Pure water has a water potential of zero. Adding solutes to pure water lowers its water potential — so the water potential of any solution is always negative. The more negative the water potential, the stronger the concentration of solutes in the solution.

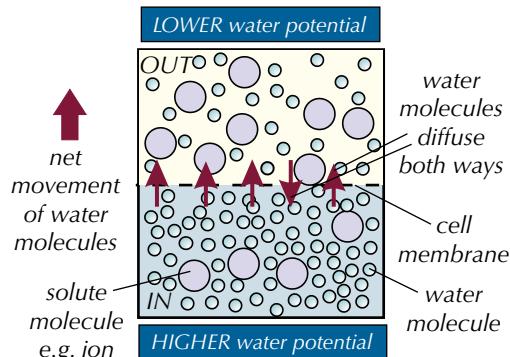


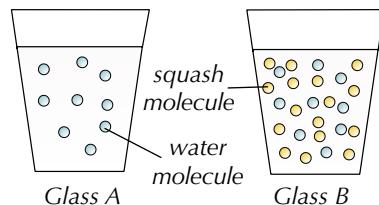
Figure 1: Osmosis across a cell membrane.

Example

Glass A contains pure water — it's got a water potential of zero.

Glass B contains a solution of orange squash. The orange squash molecules are a solute. They lower the concentration of the water molecules.

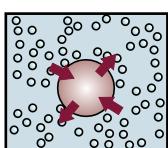
This means that the water potential of the orange squash is lower than the water potential of pure water.



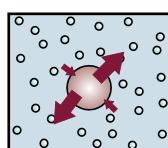
If two solutions have the same water potential they're said to be **isotonic**.

Cells in an isotonic solution won't lose or gain any water — there's no net movement of water molecules because there's no difference in water potential between the cell and the surrounding solution.

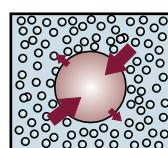
If a cell is placed in a solution that has a higher water potential it will swell as water moves into it by osmosis. Solutions with a higher water potential compared with the inside of the cell are called **hypotonic**. If a cell is placed in a solution that has a lower water potential it may shrink as water moves out of it by osmosis. Solutions with a lower water potential than the cell are called **hypertonic**.



Cell in an isotonic solution — no net movement of water.



Cell in a hypertonic solution — net movement of water out of the cell.



Cell in a hypotonic solution — net movement of water into the cell.

Factors affecting the rate of osmosis

The factors affecting the rate of osmosis are similar to those affecting the rate of diffusion (see page 102).

- The **water potential gradient** — the higher the water potential gradient, the faster the rate of osmosis. As osmosis takes place, the difference in water potential on either side of the membrane decreases, so the rate of osmosis levels off over time.
- The thickness of the exchange surface — the thinner the exchange surface, the faster the rate of osmosis.
- The surface area of the exchange surface — the larger the surface area, the faster the rate of osmosis.

Investigating water potential

You can do a simple experiment, using potato cylinders, to find out the water potential of plant tissue (see next page). There are three main steps involved:

REQUIRED
PRACTICAL 3

Tip: Before you start your investigation, make sure you do a risk assessment so you are aware of any potential hazards.

1. Making serial dilutions

Firstly you need to make up several solutions of different, known concentrations to test the cylinders in. You can do this using a **serial dilution** technique. A serial dilution is when you create a set of solutions that decrease in concentration by the same factor each time. It's a useful technique, particularly when you need to create a very weak solution, as it means you don't have to measure out very small volumes of liquid.

Example — Making serial dilutions

This is how you'd make five serial dilutions of a sucrose solution, starting with an initial sucrose concentration of 2 M and diluting each solution by a factor of 2...

1. Line up five test tubes in a rack.
2. Add 10 cm³ of the initial 2 M sucrose solution to the first test tube and 5 cm³ of distilled water to the other four test tubes (see Figure 2).
3. Then, using a pipette, draw 5 cm³ of the solution from the first test tube, add it to the distilled water in the second test tube and mix the solution thoroughly. You now have 10 cm³ of solution that's half as concentrated as the solution in the first test tube (it's 1 M).
4. Repeat this process three more times to create solutions of 0.5 M, 0.25 M and 0.125 M.

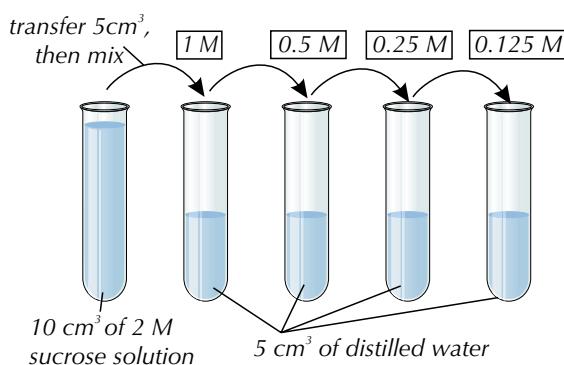


Figure 2: How to make serial dilutions.

Tip: Making serial dilutions is something you need to know how to do for your Required Practical, so you need to make sure you understand the process. See the next page for more on how to make up a solution of any given concentration.

Tip: You don't have to dilute solutions by a factor of 2. E.g. to dilute by a factor of 10, take 1 cm³ from your original sample and add it to 9 cm³ of water.

Exam Tip

Make sure you really understand what's going on here — in the exam you could get asked how you'd make up a solution of a given concentration from an initial solution of a known concentration.

Tip: You could also investigate the water potential of other plant cell types, such as carrot, using the same basic method.



Figure 3: Osmosis in carrot cells. The carrot on the left has been placed in salty water (low water potential) and the carrot on the right has been placed in pure water.

Exam Tip

Producing a calibration curve is part of this Required Practical, so make sure you can draw one and know how to use it to find the water potential of the cells you have used.

You can make solutions of any concentration by finding the scale factor.

Example — Maths Skills

If you want to make 15 cm³ of 0.4 M sucrose solution...

1. Start with a solution of a known concentration, e.g. 1 M.
2. Find the scale factor by dividing the concentration of this solution by the concentration of the solution you want to make. So in this case the scale factor = 1 M ÷ 0.4 M = **2.5**.
3. This means that the solution you want to make is 2.5 times weaker than the one you have. To make the solution 2.5 times weaker, use 2.5 times less of it, i.e. $15 \text{ cm}^3 \div 2.5 = \mathbf{6 \text{ cm}^3}$. Transfer this amount to a clean test tube.
4. Top up the test tube with distilled water to get the volume you want to make. In this case you want to make 15 cm³ of solution, so you need to add: $15 - 6 = \mathbf{9 \text{ cm}^3}$ of distilled water.

2. Measuring change in mass

Once you have made up a set of serial dilutions, you can use them to find the water potential of potato cells. First you need to measure how much mass the potato cells gain or lose in each solution...

1. Use a cork borer to cut potatoes into identically sized chips, about 1 cm in diameter. Divide the chips into groups of three and measure the mass of each group using a mass balance.
2. Place one group into each of your sucrose solutions and leave the chips in the solutions for at least 20 minutes (making sure that they all get the same amount of time).
3. Remove the chips and pat dry gently with a paper towel. Weigh each group again and record your results. Calculate the percentage change in mass for each group.

The potato chips will gain water (and therefore mass) in solutions with a higher water potential than the chips, and lose water in solutions with a lower water potential.

3. Producing a calibration curve

Next, you can use your results to produce a calibration curve by plotting percentage change in mass against the concentration of sucrose solution. You can then use your calibration curve to determine the water potential of the potato cells:

Example — Maths Skills

The point at which your calibration curve crosses the x-axis (where the percentage change in mass is 0) is the point at which the water potential of the sucrose solution is the same as the water potential of the potato cells (see Figure 4). Find the concentration at this point, then look up the water potential for that concentration of sucrose solution in, e.g. a textbook.

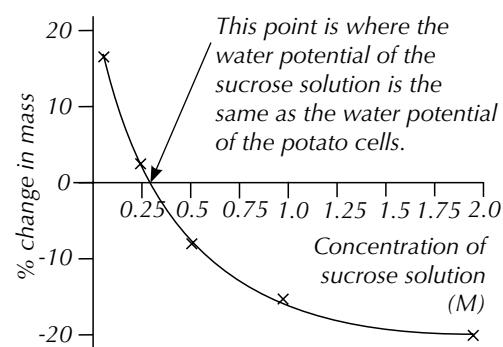


Figure 4: A calibration curve to find the water potential of potato cells.

Practice Questions — Application

- Q1 Describe the net movement of water molecules in each of the following situations:
- Human cheek cells with a water potential of -300 kPa are placed in a salt solution with a water potential of -325 kPa.
 - Apple slices with a water potential of -750 kPa are placed in a beaker of pure water.
 - Orange squash with a water potential of -450 kPa is sealed in a length of Visking tubing and suspended in a solution of equal water potential.

- Q2 Potato cells with a water potential of -350 kPa are placed in sucrose solutions with varying water potentials. The water potential of each solution is shown in the table below.

| Solution | Water potential |
|----------|-----------------|
| 1 | -250 kPa |
| 2 | -500 kPa |
| 3 | -1000 kPa |

- After 15 minutes, the potato cells in solution 1 have increased in volume. Explain why this is the case.
 - Predict whether the cells in solutions 2 and 3 will increase or decrease in volume. Explain your answers.
- Q3 A scientist has a 1.5 M saline solution. For her experiment she needs 30 cm³ of 125 mM solution.
- Calculate the volume of the original solution and distilled water that she needs to make the new solution.
 - From her 30 cm³ of 125 mM solution, she needs to make two more solutions. She will make serial dilutions, diluting by a factor of 5 each time. Describe fully how she would do this.

Tip: Water potential is usually measured in kilopascals (or kPa). It's actually a unit of pressure.

Tip: Visking tubing is a partially permeable membrane — it's used a lot in osmosis and diffusion experiments.

Tip: Remember, a higher water potential is closer to 0 (the water potential of pure water).

Tip: 1 M = 1000 mM.

Practice Questions — Fact Recall

- Define osmosis.
- Define the term 'water potential'.
- Give three factors that affect the rate of osmosis.
- Describe an investigation that you could do to find the water potential of potato cells.

Tip: You should include the use of a calibration curve in your answer to Q4.

Learning Objectives:

- Know that movement across cell membranes can occur by active transport (including the role of carrier proteins and the importance of the hydrolysis of ATP).
- Know that movement across cell membranes can occur by co-transport.
- Understand how co-transporters are involved in the absorption of sodium ions and glucose by cells lining the mammalian ileum.
- Explain how the number of carrier proteins affects the rate of movement across cell membranes.

Specification Reference 3.2.3

Tip: Most of the ATP in a cell is produced by aerobic respiration. Aerobic respiration takes place in the mitochondria and is controlled by enzymes.

Tip: Unlike facilitated diffusion, active transport doesn't use channel proteins.

Tip: It's the glucose that's being actively transported here, not the sodium ions. This is explained in more detail on the next page.

4. Active Transport

Another method of transport across a cell membrane, this time using energy...

Active transport — the basics

Active transport uses energy to move molecules and ions across plasma membranes, usually against a concentration gradient. Carrier proteins and co-transporters are involved in active transport.

Carrier proteins

The process is pretty similar to facilitated diffusion — a molecule attaches to the carrier protein, the protein changes shape and this moves the molecule across the membrane, releasing it on the other side (see Figure 1). There are two main differences between active transport and facilitated diffusion though:

- Active transport usually moves solutes from a low to a high concentration — in facilitated diffusion, they always move from a high to a low concentration.
- Active transport requires energy — facilitated diffusion does not.

ATP (a molecule produced by respiration) is a common source of energy in the cell, so it's important for active transport. ATP undergoes a hydrolysis reaction, splitting into ADP and P_i (inorganic phosphate). This releases energy so that the solutes can be transported.

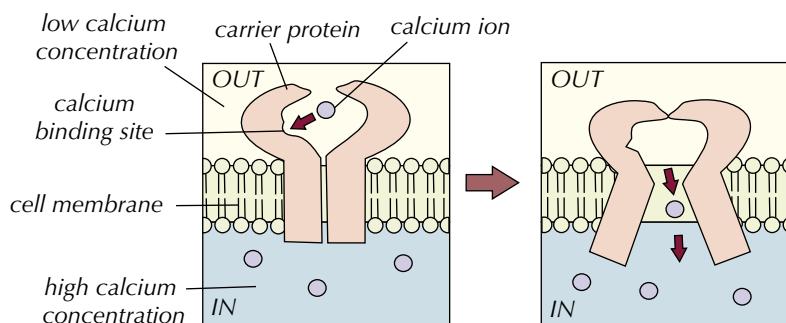


Figure 1: The active transport of calcium ions.

Co-transporters

Co-transporters are a type of **carrier protein**. They bind two molecules at a time. The concentration gradient of one of the molecules is used to move the other molecule against its own concentration gradient.

Figure 2 shows the co-transport of sodium ions and glucose. Sodium ions move across the membrane down their concentration gradient. This moves glucose across the membrane too, against its concentration gradient.

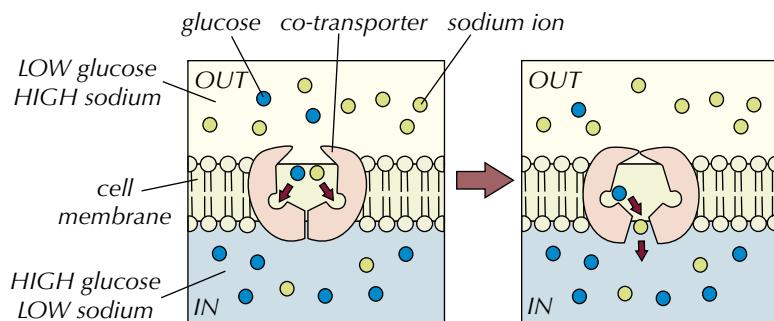


Figure 2: The co-transport of glucose and sodium ions.

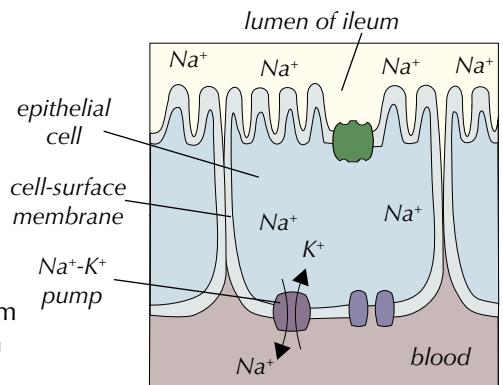
Co-transport and the absorption of glucose

Glucose is absorbed into the bloodstream in the small intestine. In the mammalian ileum (the final part of a mammal's small intestine) the concentration of glucose is too low for glucose to diffuse out into the blood. So glucose is absorbed from the lumen (middle) of the ileum by **co-transport**.

Step 1

Sodium ions are actively transported out of the epithelial cells in the ileum, into the blood, by the sodium-potassium pump.

This creates a concentration gradient — there's now a higher concentration of sodium ions in the lumen of the ileum than inside the cell.



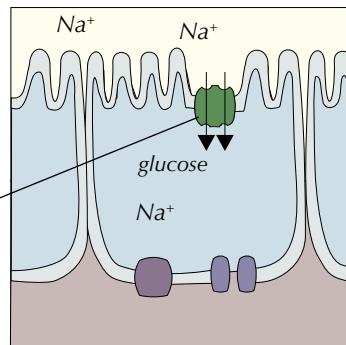
Exam Tip

You need to learn this example. Make sure you understand how co-transporters are involved in the process.

Step 2

This causes sodium ions to diffuse from the lumen of the ileum into the epithelial cell, down their concentration gradient. They do this via the sodium-glucose co-transporter proteins.

The co-transporter carries glucose into the cell with the sodium. As a result the concentration of glucose inside the cell increases.



Tip: Remember, co-transporters use the concentration gradient of one molecule (in this case the sodium ions) to move another molecule against its concentration gradient (in this case glucose).

Step 3

Glucose diffuses out of the cell, into the blood, down its concentration gradient through a protein channel, by facilitated diffusion.

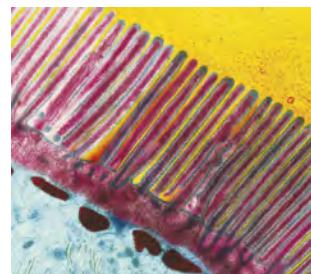
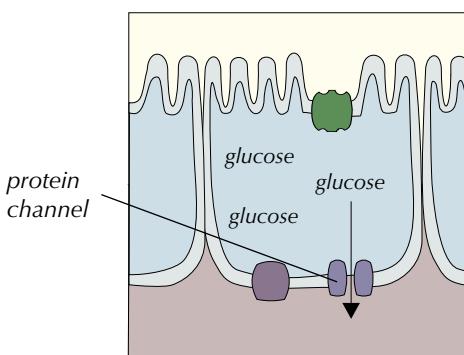


Figure 3: Coloured TEM image of microvilli (red) on epithelial cells in the small intestine. In the ileum, glucose is absorbed from the lumen (yellow) into the cytoplasm (blue) by co-transport.

As you can see from this example, the same substance can be transported into or out of a cell in different ways. Sometimes several methods of transport are needed to move a substance from A to B.

Factors affecting the rate of active transport

When active transport moves molecules and ions against their concentration gradient, a decreasing concentration gradient doesn't affect the rate of active transport. The rate of active transport is affected by:

- The speed of individual carrier proteins — the faster they work, the faster the rate of active transport.
- The number of carrier proteins present — the more proteins there are, the faster the rate of active transport.
- The rate of respiration in the cell and the availability of ATP. If respiration is inhibited, active transport can't take place.

Summary of transport mechanisms

In this section, you've covered a lot of different mechanisms that are used to transport substances across cell membranes. Here's a handy table to help you remember the similarities and differences:

| Type of transport: | Description |
|---|--|
| Diffusion (see pages 102-103) | <ul style="list-style-type: none">▪ Net movement of particles from an area of higher concentration to an area of lower concentration.▪ Passive process — doesn't require energy. |
| Facilitated diffusion (see pages 103-104) | <ul style="list-style-type: none">▪ Net movement of particles from an area of higher concentration to an area of lower concentration.▪ Uses carrier proteins and channel proteins to aid the diffusion of large molecules and charged particles through the membrane.▪ Passive process — doesn't require energy. |
| Osmosis (see pages 106-107) | <ul style="list-style-type: none">▪ Movement of water molecules across a partially permeable membrane from an area of higher water potential to an area of lower water potential.▪ Passive process — doesn't require energy. |
| Active transport (see pages 110-112) | <ul style="list-style-type: none">▪ Movement of molecules, usually from an area of lower concentration to an area of higher concentration.▪ Uses carrier proteins and co-transporters to transport molecules.▪ Active process — requires energy. |

Exam Tip

Make sure you know what types of molecules (e.g. large/small, polar/non-polar/ionic) are moved by the different types of transport.

Exam Tip

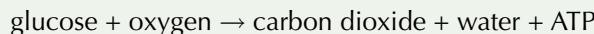
Make sure you know that active transport is the only process that uses energy.

Figure 4: Summary table of transport mechanisms.

Practice Questions — Application

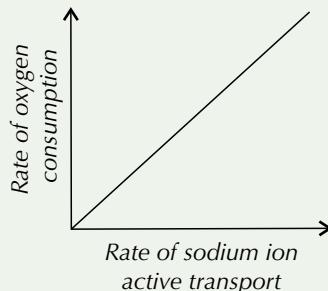
ATP is produced by mitochondria during aerobic respiration.

The overall equation for this process can be written as:



The graph below shows the relationship between the relative rates of oxygen consumption and the active transport of sodium ions across epithelial cells.

- Q1 a) Describe the relationship shown by the graph.
b) Suggest an explanation for this relationship.
c) Suggest one other factor that may affect the rate of sodium ion active transport.



- Q2 The thyroid gland needs iodide ions (I^-) to make hormones, so there is a higher concentration of I^- ions inside the thyroid cells than in the blood plasma. The Na^+/I^- co-transporter is involved in transporting I^- ions into the thyroid gland. The concentration of sodium ions is higher in the blood plasma than in the thyroid gland.
- Which ion needs to be actively transported by the Na^+/I^- co-transporter? Explain your answer.
 - Using your knowledge of co-transporters, describe and explain how active transport is carried out by the Na^+/I^- co-transporter.

Exam Tip

Questions asking you to explain graphical relationships are dead common in exams — make sure you're comfortable with doing them.

Practice Questions — Fact Recall

- Q1 Describe the chemical reaction that occurs to release energy from ATP.
- Q2 Describe how the following are used to transport substances across a cell membrane during active transport:
 - carrier proteins.
 - co-transporters.
- Q3 Why are sodium ions important in the transport of glucose from the ileum into the blood?
- Q4 Will the rate of active transport increase or decrease with an increasing number of carrier proteins?

Section Summary

Make sure you know...

- That the basic structure of cell-surface membranes (those surrounding a cell, also known plasma membranes) and internal cell membranes (e.g. those surrounding many organelles in eukaryotic cells) is the same.
- That the fluid mosaic model describes the structure of a cell membrane — this includes phospholipids arranged in a bilayer with proteins, glycoproteins, glycolipids and sometimes cholesterol scattered throughout.
- That the phospholipids in a cell membrane are always moving, and that some proteins in a membrane move sideways and some proteins are fixed in position.
- How the arrangement of phospholipids in a cell membrane forms a barrier to water-soluble substances.
- That cholesterol in a cell membrane gives the membrane stability.
- How to investigate the effect of a variable, such as temperature or solvent concentration, on cell membrane permeability, e.g. by using cubes of beetroot (Required Practical 4).
- That diffusion is the net movement of particles from an area of higher concentration to an area of lower concentration and is a passive process.
- That the phospholipid bilayer allows small, nonpolar molecules to diffuse directly through a cell membrane — this is called simple diffusion.
- How the rate of simple diffusion of a particle across a cell membrane is affected by the concentration gradient of the particle and the thickness and surface area of the membrane.
- How some specialised cells are adapted for rapid transport across their membranes. For example, cells in the small intestine have microvilli which increase their surface area for rapid diffusion.
- That facilitated diffusion is a passive process that transports large molecules (via carrier proteins) and charged particles (via channel proteins) down a concentration gradient across a cell membrane.
- How the rate of facilitated diffusion of a particle across a cell membrane is affected by the concentration gradient of the particle and the number of channel or carrier proteins in the membrane.
- That some cells are adapted for rapid facilitated diffusion across their membranes by having more transport proteins in their membranes (e.g. some kidney cells have lots of aquaporins).
- That osmosis is the diffusion of water molecules across a partially permeable membrane from an area of higher water potential to an area of lower water potential.
- How the rate of osmosis across a cell membrane is affected by the water potential gradient and the thickness and surface area of the membrane.
- How to investigate the water potential of plant tissue by producing serial dilutions of a given scale factor, placing samples of the plant tissue in the solutions and then producing a calibration curve to find the water potential of the tissue, e.g. producing serial dilutions of sucrose solution and measuring the change in mass of potato cylinders that have been immersed in the solutions (Required Practical 3).
- That active transport usually moves solutes from a low to a high concentration and requires energy (from ATP).
- That ATP releases energy during a hydrolysis reaction, in which it splits into ADP and P_i.
- That active transport, like facilitated diffusion, uses carrier proteins to transport molecules across the membrane.
- That co-transporters bind two molecules at a time, enabling the concentration gradient of one of the molecules to transport the other molecule against its concentration gradient.
- How sodium ions and glucose are absorbed by cells in a mammal's ileum by co-transport.
- That the rate of active transport is affected by the number of carrier proteins in a cell membrane.

Exam-style Questions

1

Figures 1 and 2 show onion cells under a light microscope. The cytoplasm appears dark grey. One of the figures shows the onion cells after they have been placed in a weak salt solution. The solution has a lower water potential than the onion cells.

Figure 1

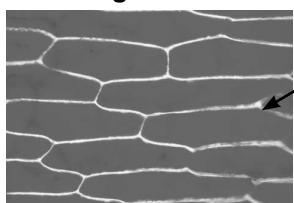
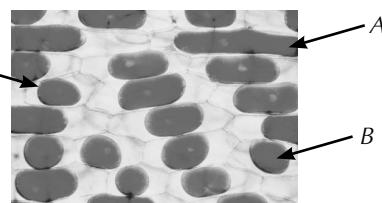


Figure 2



- 1.1 Which figure shows the cells after they have been placed in the salt solution? Explain your answer.

(2 marks)

- 1.2 Look at the cells labelled **A** and **B** on **Figure 2**. By comparing the size of their membranes, suggest which of these cells is most likely to experience the fastest transport of water molecules into and out of its cytoplasm. Explain your answer.

(1 mark)

- 1.3 The cells' surface membranes contain phospholipids. Describe the arrangement of the phospholipids based on the fluid mosaic model of membrane structure.

(2 marks)

- 1.4 The cell membranes also contain proteins. Some of the proteins have carbohydrates attached. What name is given to these molecules?

(1 mark)

- 1.5 An onion cell membrane contains less cholesterol than an animal cell membrane. Suggest and explain why this is.

(2 marks)

2

Glucose is a product of digestion. It is also a relatively large polar molecule. Once glucose has been digested, it must be absorbed into the bloodstream from the cells of the ileum. Part of the absorption process happens using active transport.

- 2.1 Explain what is meant by the term active transport.

(2 marks)

- 2.2 State the type of molecule that actively transports glucose across the cell-surface membranes of the ileum and briefly describe how it does so.

(2 marks)

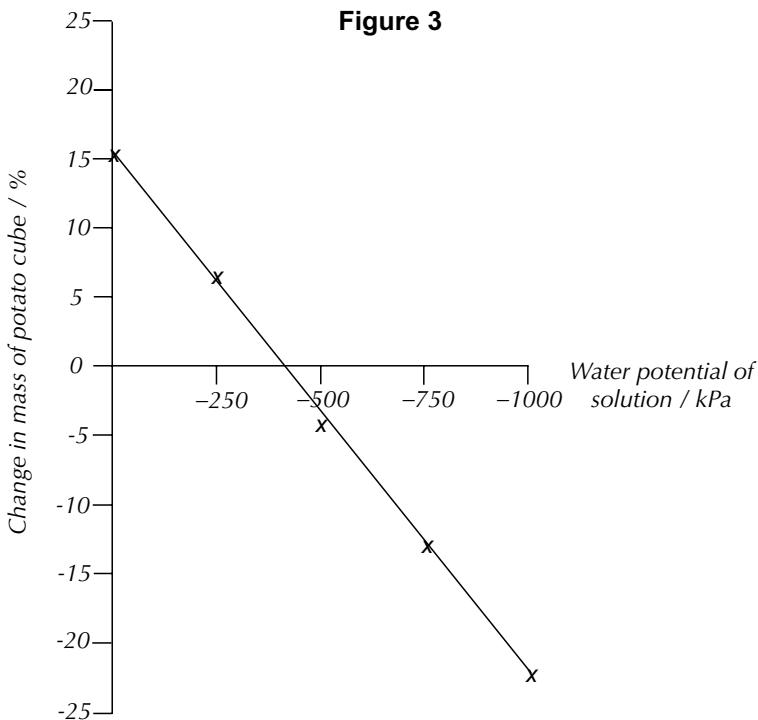
- 2.3 Another stage of the absorption process happens by facilitated diffusion. Suggest why glucose must use facilitated diffusion rather than simple diffusion to cross the cell-surface membranes of the ileum.

(3 marks)

3

A group of students investigated the water potential of potato cells.

They cut cubes of potato of equal size and shape, weighed them and placed a single cube into one of four different concentrations of sucrose solution. One cube was placed in pure water. They re-weighed each of the cubes every hour and after 12 hours the mass of all the cubes remained constant. The overall change in mass for each cube is shown in **Figure 3**.



- 3.1 The students recorded the difference in mass between the cubes at the start and end of the experiment in grams, but plotted the overall change as a percentage. Suggest why the graph was plotted in this way.

(1 mark)

- 3.2 What was the change in mass for the potato cube placed in pure water?

(1 mark)

- 3.3 Explain why the cubes in the -500 , -750 and -1000 kPa solutions lost mass.

(2 marks)

- 3.4 Use **Figure 3** to estimate the water potential of the potato cells.

(1 mark)

- 3.5 Suggest how the students could make their results more precise.

(1 mark)

- 3.6 If the experiment was repeated with cubes that had a larger surface area would you expect the mass of all the cubes to become constant before 12 hours, at 12 hours or after 12 hours? Explain your answer.

(2 marks)

1. Antigens

Cell-surface membranes contain proteins that act as antigens. These antigens allow the immune system to tell the difference between your own, healthy body cells (known as 'self' cells) and 'foreign' invaders...

What are antigens?

Antigens are molecules (usually proteins) that can generate an **immune response** when detected by the body — see pages 118-121. They are usually found on the surface of cells, including all your body cells. Antigens that aren't normally found in the body are referred to as **foreign antigens** — it's these antigens that the immune system usually responds to. Antigens allow the immune system to identify:

Pathogens

These are organisms that cause disease, e.g. bacteria, viruses and fungi. All pathogens have antigens on their surface — these are identified as foreign by immune system cells, which then respond to destroy the pathogen.

Abnormal body cells

Cancerous or pathogen-infected cells have abnormal antigens on their surface, which trigger an immune response.

Toxins

These are poisons. They're also molecules, not cells. Some toxins are produced by bacteria, e.g. the bacterium *Clostridium botulinum* releases a protein toxin that affects the nervous system, causing the symptoms of botulism. The immune system can respond to toxins, as well as the pathogens that release them.

Learning Objectives:

- Recall the definition of an antigen.
- Know that each type of cell has specific molecules (antigens) on its surface that identify it.
- Understand that these molecules include proteins and enable the immune system to identify pathogens, cells from other organisms of the same species, abnormal body cells and toxins.

Specification Reference 3.2.4

Tip: The toxin itself is an antigen — it doesn't have antigens on its surface.

Tip: The ABO blood groups are A, B, AB and O. Type A blood has A antigens on its red blood cells, type B blood has B antigens and type AB blood has both A and B antigens. Type O blood doesn't have any A or B antigens. So if a person has type B blood, for example, their immune system won't recognise type A antigens on blood cells from other people.

Practice Questions — Fact Recall

- Q1 What are antigens?
Q2 Why do some antigens generate an immune response?

Learning Objectives:

- Recall the process of phagocytosis of pathogens and the subsequent destruction of ingested pathogens by lysozymes.
- Know the response of T lymphocytes to a foreign antigen (the cellular response).
- Understand the role of antigen-presenting cells in the cellular response.
- Understand the role of helper T-cells in stimulating cytotoxic T-cells, B cells and phagocytes.
- Know the response of B lymphocytes to a foreign antigen (the humoral response), including clonal selection and the release of monoclonal antibodies.
- Recall the definition of an antibody and know its general structure.
- Understand how an antigen-antibody complex is formed, leading to the destruction of the antigen by agglutination and phagocytosis of bacterial cells.
- Understand the roles of plasma cells and of memory cells in producing primary and secondary immune responses.

Specification
Reference 3.2.4

2. The Immune Response

There's an army of cells in the body that helps to protect us from disease — together, they're called the immune system.

The main stages of the immune response

1. Phagocytosis

A **phagocyte** (e.g. a macrophage) is a type of white blood cell that carries out phagocytosis (engulfment of pathogens). They're found in the blood and in tissues and are the first cells to respond to an immune system trigger inside the body. Here's how they work:

- A phagocyte recognises the foreign antigens (see previous page) on a pathogen.
- The cytoplasm of the phagocyte moves round the pathogen, engulfing it.
- The pathogen is now contained in a **phagocytic vacuole** (a bubble) in the cytoplasm of the phagocyte.
- A **lysosome** (an organelle that contains enzymes called **lysozymes**) fuses with the phagocytic vacuole. The lysozymes break down the pathogen.
- The phagocyte then presents the pathogen's antigens — it sticks the antigens on its surface to activate other immune system cells. The phagocyte is acting as an antigen-presenting cell.

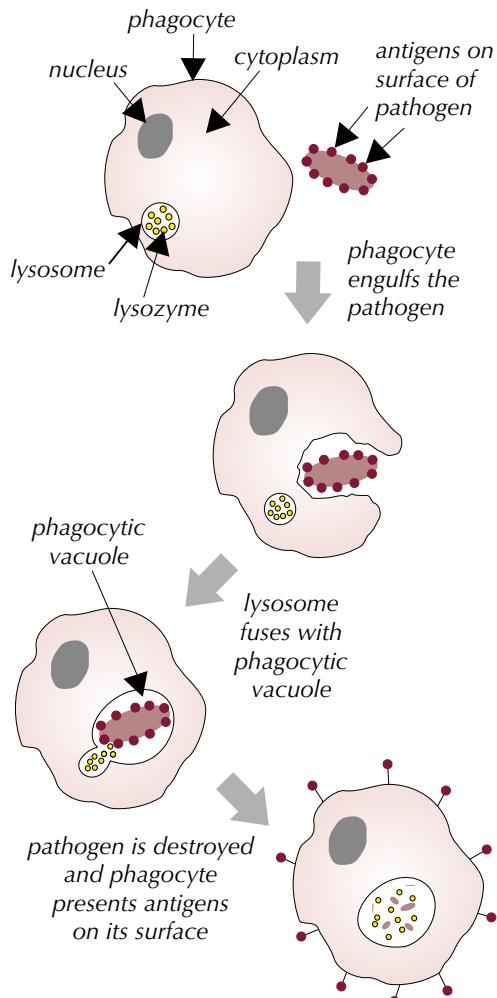


Figure 1: Phagocytosis and antigen presentation.

2. T-Cells

A **T-cell** (also called a T-lymphocyte) is another type of white blood cell. It has receptor proteins on its surface that bind to complementary antigens presented to it by phagocytes. This activates the T-cell.

Different types of T-cells respond in different ways. For example, **helper T-cells** (T_H cells) release chemical signals that activate and stimulate phagocytes and **cytotoxic T-cells** (T_C cells), which kill abnormal and foreign cells. T_H cells also activate **B-cells**, which secrete antibodies (see next page).

3. B-Cells

B-cells (also called B-lymphocytes) are also a type of white blood cell. They're covered with antibodies — proteins that bind to antigens to form an **antigen-antibody complex**. Each B-cell has a different shaped antibody on its membrane, so different ones bind to different shaped antigens (see Figure 2).

Tip: B-cells also have receptor proteins on their surface that bind to the signalling molecules released by the T_H cells.

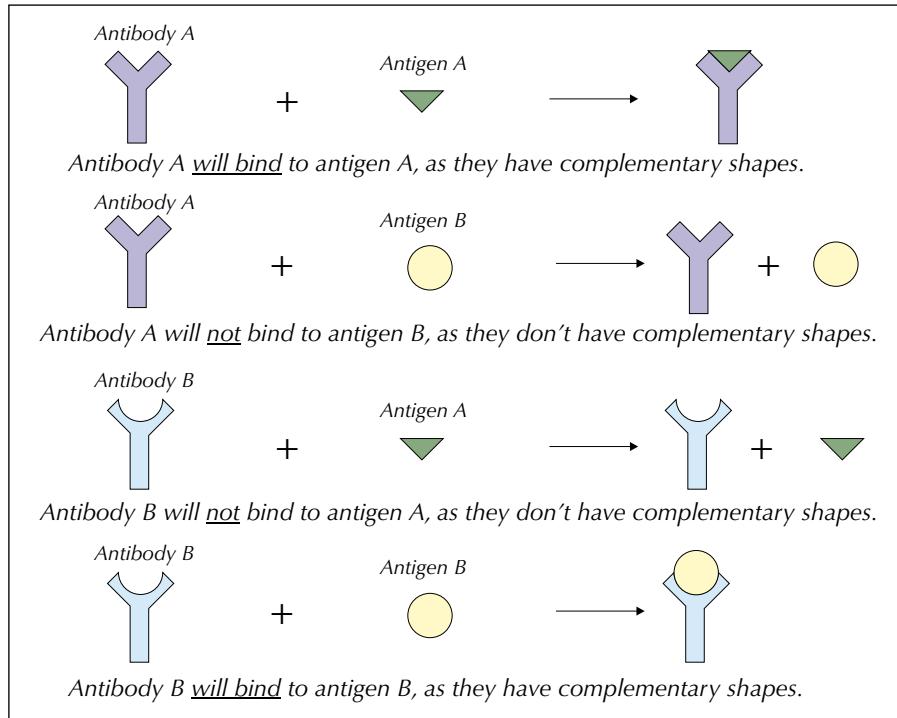


Figure 2: Complementary binding between antibodies and antigens.

When the antibody on the surface of a B-cell meets a complementary shaped antigen, it binds to it. This, together with substances released from helper T-cells, activates the B-cell. This process is called **clonal selection**. The activated B-cell divides into **plasma cells**.

4. Antibody production

Plasma cells are identical to the B-cell (they're clones). They secrete loads of antibodies specific to the antigen. These are called **monoclonal antibodies**. They bind to the antigens on the surface of the pathogen to form lots of **antigen-antibody complexes** (see Figure 5 on the next page).

An antibody has two binding sites, so can bind to two pathogens at the same time. This means that pathogens become clumped together — this is called **agglutination**. Phagocytes then bind to the antibodies and phagocytose many pathogens at once. This process leads to the destruction of pathogens carrying this antigen in the body.

You need to learn the general structure of an antibody for your exam — this is also shown in Figure 5. Antibodies are proteins — they're made up of chains of amino acids. The specificity of an antibody depends on its **variable regions**, which form the antigen binding sites. Each antibody has a variable region with a unique tertiary structure (due to different amino acid sequences) that's complementary to one specific antigen. All antibodies have the same **constant regions**.

Tip: Antibodies bind to antigens because they have a complementary shape — like a lock fits a key.

Exam Tip
Never say that antigens and antibodies have the 'same shape' or a 'matching shape' — you need to use the phrase 'complementary shape'.

Tip: The B-cell divides by mitosis, so that all the cells produced are genetically identical. This means that they all produce identical (monoclonal) antibodies specific to the pathogen.

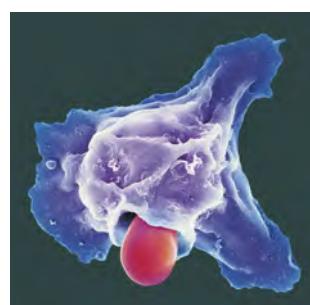


Figure 3: An electron micrograph of a phagocyte (blue) engulfing a pathogen (red).



Figure 4: A molecular model of an antibody.

Tip: The tertiary structure of a protein is the folding of the amino acid chain into the protein's 3D shape. See page 34 for more on protein structure.

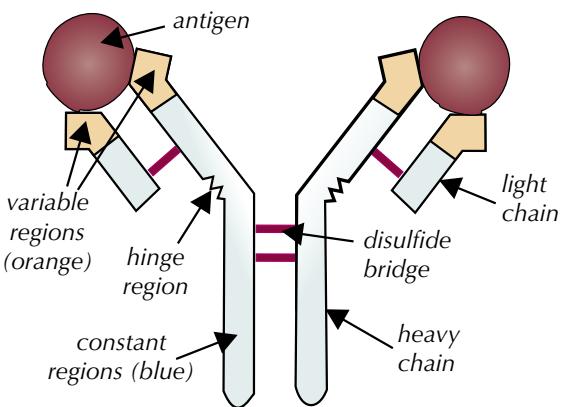


Figure 5: Antigen-antibody complex and antibody structure.

Cellular and humoral responses

Just to add to your fun, the immune response is split into two — the cellular response and the humoral response.

- Cellular — The T-cells and other immune system cells that they interact with, e.g. phagocytes, form the cellular response.
- Humoral — B-cells, clonal selection and the production of monoclonal antibodies form the humoral response.

Both types of response are needed to remove a pathogen from the body and the responses interact with each other, e.g. T-cells help to activate B-cells, and antibodies coat pathogens making it easier for phagocytes to engulf them.

Primary and secondary immune responses

The primary response

When an antigen enters the body for the first time it activates the immune system. This is called the primary response. The primary response is slow because there aren't many B-cells that can make the antibody needed to bind to it. Eventually the body will produce enough of the right antibody to overcome the infection. Meanwhile the infected person will show symptoms of the disease.

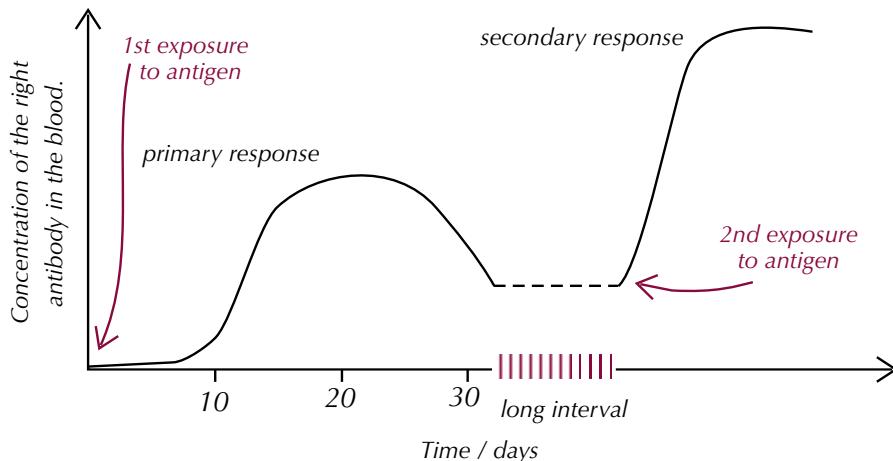
After being exposed to an antigen, both T- and B-cells produce **memory cells**. These memory cells remain in the body for a long time. Memory T-cells remember the specific antigen and will recognise it a second time round. Memory B-cells record the specific antibodies needed to bind the antigen. The person is now immune — their immune system has the ability to respond quickly to a second infection.

The secondary response

If the same pathogen enters the body again, the immune system will produce a quicker, stronger immune response — the secondary response. Clonal selection happens faster. Memory B-cells are activated and divide into plasma cells that produce the right antibody to the antigen. Memory T-cells are activated and divide into the correct type of T-cells to kill the cell carrying the antigen. The secondary response often gets rid of the pathogen before you begin to show any symptoms (see Figure 6 on the next page).

Tip: Being immune doesn't mean you'll never be infected by that pathogen again, it just means that if it gets into your body a second time your immune system quickly kills it before you get ill.

Tip: The secondary response only happens if it's the same pathogen. If it's a different pathogen you just get another primary response.

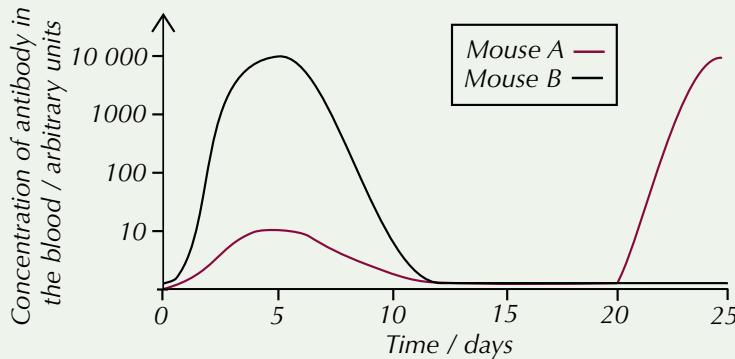


Tip: The secondary response is always faster than the primary response. This is shown by a steeper line in graphs of blood antibody concentration against time.

Figure 6: A graph of antibody concentration against time since antigen exposure.

Practice Questions — Application

- Q1 The graph below shows the immune responses of two mice exposed to a pathogen. Both mice were exposed on day 0 of the experiment.



- How much antibody did each mouse have in its blood on day 5?
 - Which mouse was already immune to the pathogen?
Explain your answer.
 - i) On which day was Mouse A exposed to the pathogen again?
ii) Describe what happened to Mouse A's immune system after it was exposed again.
- Q2 Rheumatic fever is a disease where the immune system attacks cells in the heart. It's often triggered by an infection with the bacterium *Streptococcus pyogenes*. Antigens on the surface of *S. pyogenes* have a very similar shape to antigens on the surface of heart cells.
Suggest why *S. pyogenes* infection can lead to rheumatic fever.

Practice Questions — Fact Recall

- What is the function of helper T-cells?
- What is the function of plasma cells?
- What is the difference between the cellular immune response and the humoral immune response?
- Give three differences (other than speed) between a primary and a secondary immune response.

Learning Objectives:

- Know the differences between active and passive immunity.
- Understand the use of vaccines to provide protection for individuals and populations against disease.
- Understand the concept of herd immunity.
- Be able to discuss ethical issues associated with the use of vaccines.

Specification Reference 3.2.4

Exam Tip

Don't get active and passive immunity mixed up in the exam.

Just remember that in active immunity your body is actively doing something — it's producing antibodies.

Tip: A lot of vaccines contain more than one antigen from the same pathogen, to make sure it makes you immune to the pathogen. Some vaccines also contain antigens to more than one pathogen, e.g. the MMR vaccine contains antigens from the pathogens that cause measles, mumps and rubella.

3. Immunity and Vaccines

After you've been infected once by a pathogen you'll be immune to it, but being infected in the first place can be pretty unpleasant. Vaccination can make you immune without the being ill part.

Active and passive immunity

Immunity can be active or passive:

Active immunity

This is the type of immunity you get when your immune system makes its own antibodies after being stimulated by an antigen. There are two different types of active immunity:

1. **Natural** — this is when you become immune after catching a disease.
2. **Artificial** — this is when you become immune after you've been given a vaccination containing a harmless dose of antigen (see below).

Passive immunity

This is the type of immunity you get from being given antibodies made by a different organism — your immune system doesn't produce any antibodies of its own. Again, there are two types:

1. **Natural** — this is when a baby becomes immune due to the antibodies it receives from its mother, through the placenta and in breast milk.
2. **Artificial** — this is when you become immune after being injected with antibodies from someone else. E.g. If you contract tetanus you can be injected with antibodies against the tetanus toxin, collected from blood donations.

In the exam you might be asked about the differences between these types of immunity:

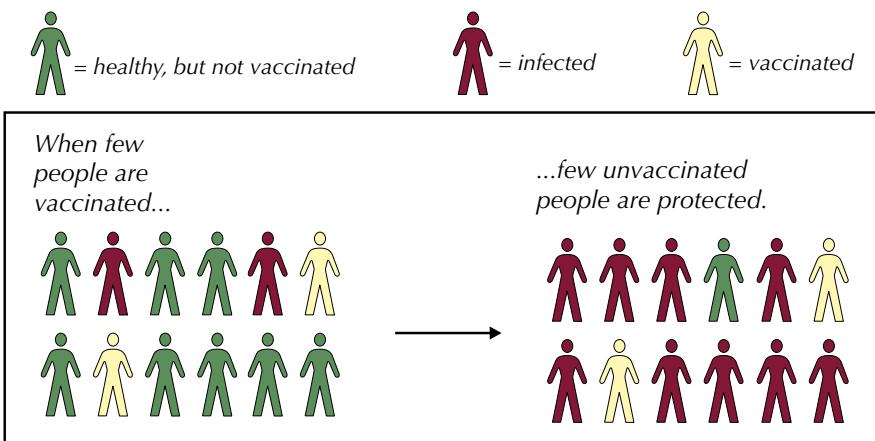
| Active Immunity | Passive Immunity |
|--|---|
| Requires exposure to antigen | Doesn't require exposure to antigen |
| It takes a while for protection to develop | Protection is immediate |
| Memory cells are produced | Memory cells aren't produced |
| Protection is long-term because the antibody is produced (after activation of memory cells) in response to complementary antigen being present in the body | Protection is short-term because the antibodies given are broken down |

Vaccination

While your B-cells are busy dividing to build up their numbers to deal with a pathogen (i.e. the primary response — see page 120), you suffer from the disease. Vaccination can help avoid this.

Vaccines contain antigens that cause your body to produce memory cells against a particular pathogen, without the pathogen causing disease. This means you become immune without getting any symptoms.

Vaccines protect individuals that have them and, because they reduce the occurrence of the disease, those not vaccinated are also less likely to catch the disease (because there are fewer people to catch it from). This is called **herd immunity** — see Figure 1 on the next page.



Tip: Vaccinations are sometimes called immunisations.

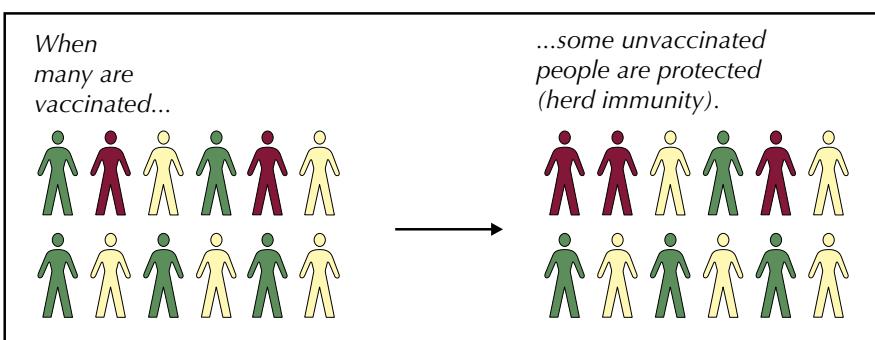


Figure 1: Herd immunity

Vaccines always contain antigens — these may be free or attached to a dead or attenuated (weakened) pathogen. Vaccines may be injected or taken orally. The disadvantages of taking a vaccine orally are that it could be broken down by enzymes in the gut or the molecules of the vaccine may be too large to be absorbed into the blood. Sometimes booster vaccines are given later on (e.g. after several years) to make sure that more memory cells are produced.

Tip: Attenuated viruses have usually been genetically or chemically modified so that they can't produce toxins or attach to and infect host cells.

Ethical issues surrounding the use of vaccines

All vaccines are tested on animals before being tested on humans — some people disagree with animal testing. Also, animal based substances may be used to produce a vaccine, which some people disagree with.

Testing vaccines on humans can be risky, e.g. volunteers may put themselves at unnecessary risk of contracting the disease because they think they're fully protected (e.g. they might have unprotected sex because they have had a new HIV vaccine and think they're protected — and the vaccine might not work).

Some people don't want to take the vaccine due to the risk of side effects, but they are still protected because of herd immunity — other people think this is unfair.

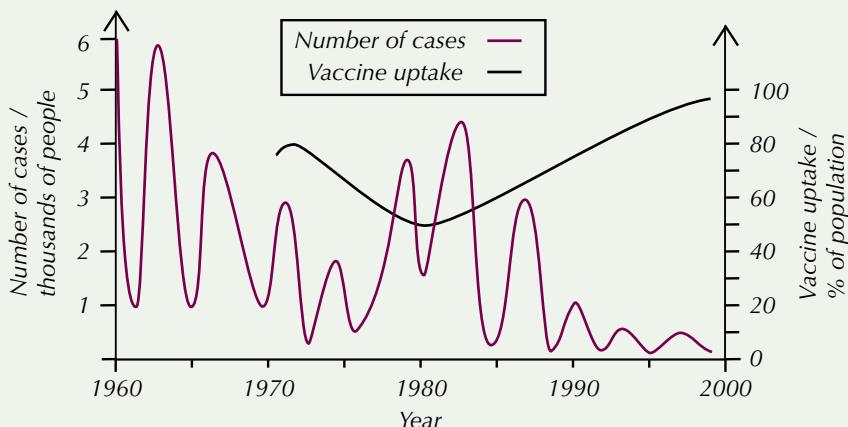
If there was an epidemic of a new disease (e.g. a new influenza virus) there would be a rush to receive a vaccine and difficult decisions would have to be made about who would be the first to receive it.

Practice Questions — Application

Whooping cough is an infection of the respiratory system. The graph below shows the number of cases of whooping cough in Scotland between 1960 and 1999, and the vaccine uptake from the 1970s to 1999.

Exam Tip

Always pay attention to the units on the axes — on the graph on the right, the y-axis is number of cases in thousands of people, so in 1963 there weren't 6 cases, there were 6000.



Exam Tip

When reading off graphs with multiple scales, double check you've got the right one. If you're struggling to read off the answer, draw lines on the graph to help you.

- Q1 What percentage of the population were vaccinated in 1990?
- Q2 How many cases of whooping cough were there in 1965?
- Q3 The whooping cough vaccine was introduced in Scotland in the 1950s. Describe and explain the overall trend in the number of cases of whooping cough between 1960 and 1975.
- Q4 In the 1970s some people were concerned that the vaccine caused neurological problems, such as seizures.
 - a) What happened to the uptake of the vaccine in the 1970s?
 - b) Explain how this change affected the number of cases between the mid 1970s and the mid-1980s.

Practice Questions — Fact Recall

- Q1 Define the terms active and passive immunity.
- Q2 How do vaccines give people immunity?
- Q3 What is herd immunity?
- Q4 Describe two issues surrounding the use of vaccinations.

4. Antigenic Variation

Just to complicate things, pathogens can change their antigens to trick the immune system.

What is antigenic variation?

Antigens on the surface of pathogens activate the primary response. When you're infected a second time with the same pathogen (which has the same antigens on its surface) they activate the secondary response and you don't get ill.

However, some sneaky pathogens can change their surface antigens. This is called **antigenic variation**. (Different antigens are formed due to changes in the genes of a pathogen.) This means that when you're infected for a second time, the memory cells produced from the first infection will not recognise the different antigens. So the immune system has to start from scratch and carry out a primary response against these new antigens. This primary response takes time to get rid of the infection, which is why you get ill again.

Antigenic variation also makes it difficult to develop vaccines against some pathogens for the same reason. Examples of pathogens that show antigenic variation include HIV and the influenza virus. Here's how antigenic variation affects the production of vaccines to help prevent people catching influenza:

Example

The influenza (flu) vaccine changes every year. That's because the antigens on the surface of the influenza virus change regularly, forming new strains of the virus.

Memory cells produced from vaccination with one strain of the flu will not recognise other strains with different antigens. The strains are immunologically distinct. Every year there are different strains of the influenza virus circulating in the population, so a different vaccine has to be made.

New vaccines are developed and one is chosen every year that is the most effective against the recently circulating influenza viruses. Governments and health authorities then implement a programme of vaccination using the most suitable vaccine.

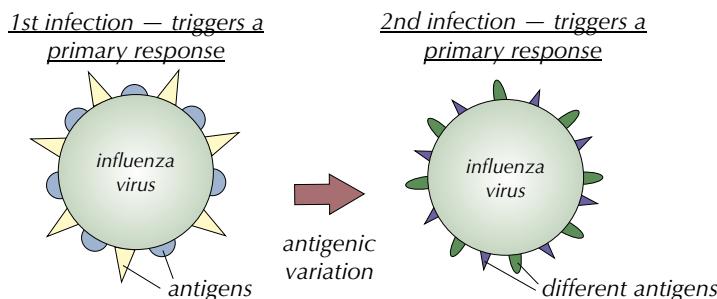


Figure 2: Antigenic variation in the influenza virus.

Learning Objective:

- Understand the effect of antigen variability on disease and disease prevention.

Specification Reference 3.2.4

Tip: Pathogens of the same type that show antigenic variation are often referred to as strains.

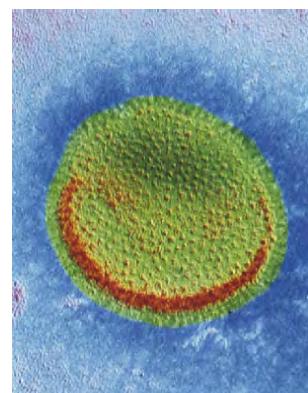


Figure 1: A TEM of an influenza virus.

Practice Questions — Fact Recall

Q1 What is antigenic variation?

Q2 Explain why you can become ill with flu even if you've been infected by the influenza virus before.

Learning Objectives:

- Understand the use of monoclonal antibodies in targeting medication to specific cell types by attaching a therapeutic drug to an antibody, and in medical diagnosis.
- Understand the use of antibodies in the ELISA test.
- Be able to discuss ethical issues associated with the use of monoclonal antibodies.

Specification Reference 3.2.4

Tip: The unique tertiary structure of the antibody binding sites is due to the unique order of the amino acids in the protein (its primary structure — see p. 34).

Tip: Anti-cancer drugs are basically toxic chemicals that kill cells — they cause side effects because they also kill cells that aren't cancerous. Targeting the drugs using antibodies helps reduce this problem.

Tip: The steps in this Example are illustrated in Figure 2 on the next page.

5. Antibodies in Medicine

Scientists can make antibodies in the lab and use them for all sorts of stuff...

The use of monoclonal antibodies

Monoclonal antibodies are antibodies produced from a single group of genetically identical B-cells (plasma cells). This means that they're all identical in structure.

As you know, antibodies are very specific because their binding sites have a unique tertiary structure (see p. 34) that only an antigen with a complementary shape can fit into. You can make monoclonal antibodies that bind to anything you want, e.g. a cell antigen or other substance, and they will only bind to (target) this molecule. This can be useful for both treating illnesses and in medical diagnosis.

Example — Anti-cancer drugs targeted to cancer cells

Different cells in the body have different surface antigens.

Cancer cells have antigens called tumour markers that are not found on normal body cells. Monoclonal antibodies can be made that will bind to the tumour markers. You can also attach anti-cancer drugs to the antibodies. When the antibodies come into contact with the cancer cells they will bind to the tumour markers. This means the drug will only accumulate in the body where there are cancer cells. So, the side effects of an antibody-based drug are lower than other drugs because they accumulate near specific cells.

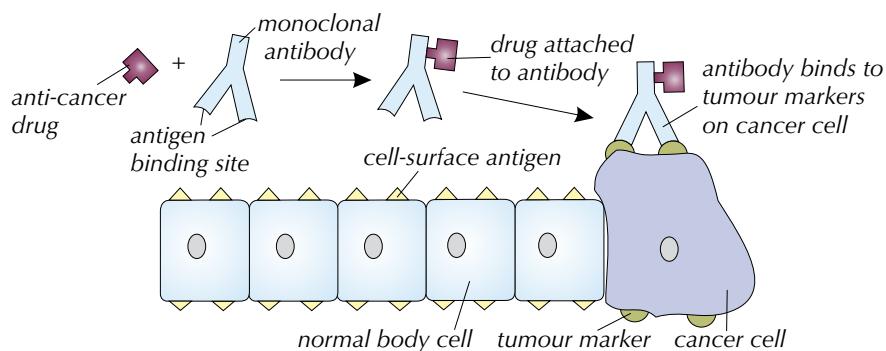


Figure 1: Targeting anti-cancer drugs to cancerous cells.

Example — Pregnancy tests

Pregnancy tests detect the hormone human chorionic gonadotropin (hCG) that's found in the urine of pregnant women:

- The application area contains antibodies that are complementary to the hCG protein, bound to a coloured bead (blue).
- When urine is applied to the application area any hCG will bind to the antibody on the beads, forming an antigen-antibody complex.
- The urine moves up the stick to the test strip, carrying any beads with it.
- The test strip contains antibodies to hCG that are stuck in place (immobilised).
- If there is hCG present the test strip turns blue because the immobilised antibody binds to any hCG — concentrating the hCG-antibody complex with the blue beads attached. If no hCG is present, the beads will pass through the test area without binding to anything, and so it won't go blue.

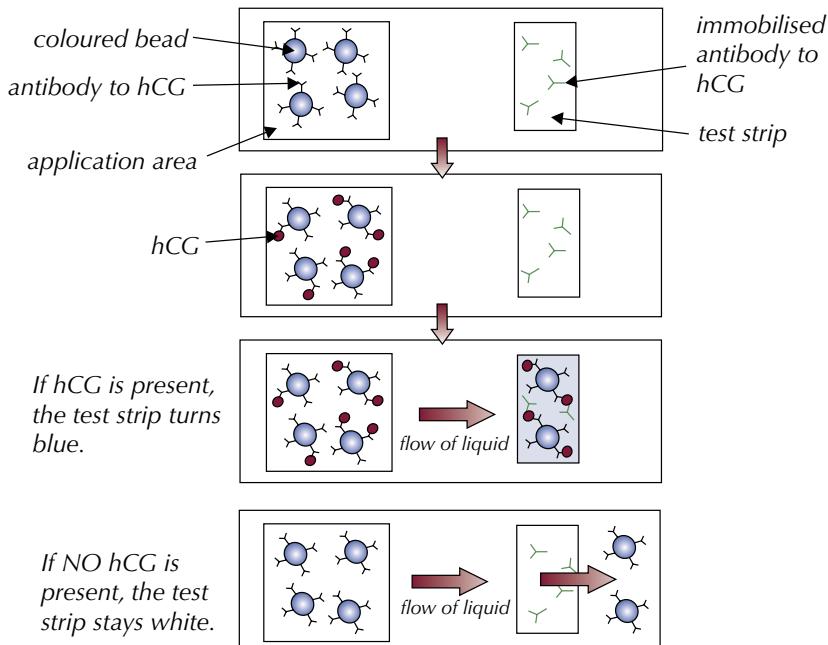


Figure 2: How a pregnancy test works.



Figure 3: A lab technician testing a urine sample for hCG.

You can use similar test strips in other areas of medical diagnosis, e.g. malaria can be diagnosed using a test strip that detects malaria antigens in the blood.

ELISA test

The enzyme-linked immunosorbent assay (ELISA) allows you to see if a patient has any antibodies to a certain antigen or any antigen to a certain antibody. It can be used in medical diagnosis to test for pathogenic infections (e.g. for HIV infection), for allergies (e.g. to nuts or lactose) and for just about anything you can make an antibody for.

In an ELISA test, an antibody is used which has an enzyme attached to it. This enzyme can react with a substrate to produce a coloured product. This causes the solution in the reaction vessel to change colour.

If there's a colour change, it demonstrates that the antigen or antibody of interest is present in the sample being tested (e.g. blood plasma). In some types of ELISA, the quantity of this antigen/antibody can be worked out from the intensity of the colour change.

There are several different types of ELISA — the simplest is the direct ELISA.

Direct ELISA

A direct ELISA uses a single antibody that is complementary to the antigen you're testing for.

Antigens from a patient sample are bound to the inside of a well in a well plate (a plastic tray with loads of little circular pits in it). A detection antibody (with an attached enzyme) that is complementary to the antigen of interest is added. If the antigen of interest is present in the patient sample, it will be immobilised on the inside surface of the well and the detection antibody will bind to it — see Figure 4 on the next page. The well is then washed out to remove any unbound antibody and a substrate solution is added. If the detection antibody is present, the enzyme reacts with the substrate to give a colour change. This is a positive result for presence of the antigen.

Tip: An allergy is an inappropriate reaction of the immune system to an antigen that shouldn't normally trigger a response, e.g. a protein in food.

Tip: The intensity of the colour change can be measured by reading the absorbance of the solution (how much light it absorbs). This absorbance value can then be compared to the absorbance of a known concentration of antibody or antigen to work out the concentration in the sample.

Tip: The colour change you see varies depending on the enzyme and substrate that are used.

Tip: A person is only likely to have antibodies to HIV if they're infected with HIV. An exception to this is babies born to mothers with HIV — see page 134.

Tip: The washing steps are important to make sure unbound antibodies aren't left in the well which could affect the results. E.g. unbound secondary antibodies could cause the test to appear positive when there are no HIV antibodies present.

Tip: If the ELISA result was negative, there would be no colour change because there would be no HIV-specific antibodies for the secondary antibodies to bind to.

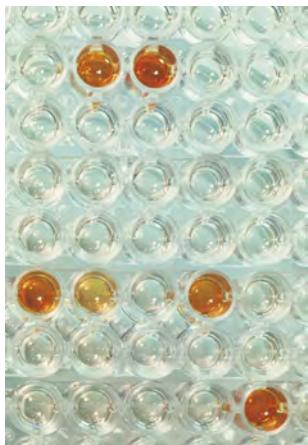


Figure 5: ELISA test results for HIV. The coloured wells show a positive result.

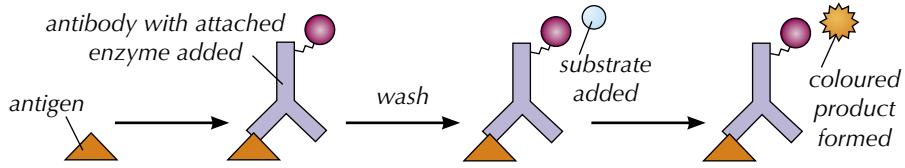


Figure 4: A direct ELISA test.

Indirect ELISA

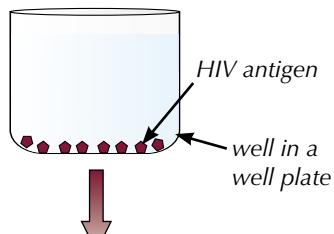
Indirect ELISA is different because it uses two different antibodies. This method is outlined in the example below:

Example

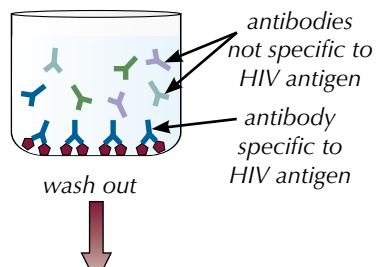
An indirect ELISA test can be used to see if a patient possesses antibodies to HIV (Human Immunodeficiency Virus):

1. HIV antigen is bound to the bottom of a well in a well plate.
2. A sample of the patient's blood plasma, which might contain several different antibodies, is added to the well. If there are any HIV-specific antibodies in the plasma (i.e. antibodies against HIV) these will bind to the HIV antigen stuck to the bottom of the well. The well is then washed out to remove any unbound antibodies.
3. A secondary antibody, that has a specific enzyme attached to it, is added to the well. This secondary antibody can bind to the HIV-specific antibody (which is also called the primary antibody). The well is washed out again to remove any unbound secondary antibody. If there's no primary antibody in the sample, all of the secondary antibody will be washed away because there will be nothing for it to bind to.
4. A solution is added to the well. This solution contains a substrate, which is able to react with the enzyme attached to the secondary antibody and produce a coloured product. If the solution changes colour, it indicates that the patient has HIV-specific antibodies in their blood and is infected with HIV.

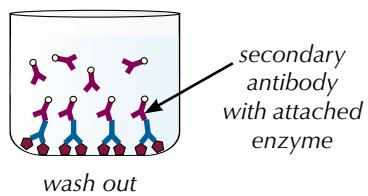
1. HIV antigen bound to the bottom of a well



2. Plasma sample added



3. Secondary antibody added



4. Substrate added

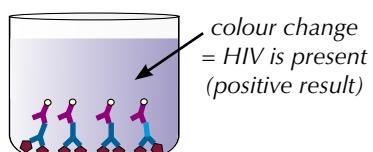


Figure 6: Stages in an ELISA test for HIV. A positive result is shown.

Ethical issues surrounding the use of monoclonal antibodies

Ethical issues surrounding monoclonal antibody therapy often involve animal rights issues. Animals are used to produce the cells from which the monoclonal antibodies are produced. Some people disagree with the use of animals in this way.

Practice Questions — Application

Q1 Donated blood is tested to see which type it is. There are four main blood types — A (containing antigen A), B (containing antigen B), AB (containing antigens A and B) and type O (containing neither).

The blood type test can be done using monoclonal antibodies produced against these antigens. The table below shows the results for four people.

| Person | Result with anti-antigen A | Result with anti-antigen B |
|--------|----------------------------|----------------------------|
| 1 | Positive — binding occurs | Negative — no binding |
| 2 | Positive — binding occurs | Positive — binding occurs |
| 3 | Negative — no binding | Negative — no binding |
| 4 | Negative — no binding | Positive — binding occurs |

- a) What blood type is:
 - i) Person 1? ii) Person 3?
 - b) People with blood type B carry anti-antigen A antibodies in their blood. If these antibodies meet antigen A the blood clots, which can kill the patient. Could they accept blood from:
 - i) Person 2? ii) Person 4?
- Q2 A scientist is using an indirect ELISA to test a patient for an allergy to gluten. First she coats a well plate with gluten protein. She then adds the patient serum sample to three of the wells. She then washes the well plate and adds a secondary antibody that has an attached enzyme. The scientist washes the well plate again, then adds a substrate solution.
- a) Why is the well plate washed out after the secondary antibody is added?
 - b) The substrate will change colour if it reacts with the enzyme bound to the secondary antibody. What would you expect the scientist to observe if the patient is allergic to the gluten protein? Explain your answer.
 - c) Suggest why the scientist adds the patient serum sample to more than one well.
 - d) Two control wells are used in this test. In one well, antibodies specific to the gluten protein are used instead of patient serum. In the other well, a salt solution is used instead of patient serum. Suggest what each of these controls are designed to show. State what you would expect the result of the test to be in each case.

Tip: Antibody names can get a bit confusing — usually, whatever comes after ‘anti-’ will be what the antibody will bind to.

Tip: Serum is the liquid remaining when all the blood cells and clotting agents have been removed from a blood sample. Proteins such as antibodies remain in the serum.

Tip: A control is an experiment designed to either check that only the independent variable is affecting the dependent variable, or to check that a positive result is possible (see p. 2 for more on controls).

Learning Objective:

- Be able to evaluate methodology, evidence and data relating to the use of vaccines and monoclonal antibodies.

Specification Reference 3.2.4

Tip: Sample size is really important in scientific studies — the bigger the better. See page 3 for more on sample size.

Tip: Bias is when someone intentionally, or unintentionally, favours a particular result.

6. Interpreting Data About Vaccines and Antibodies

When a study presents evidence for a new theory (e.g. a vaccine has a dangerous side effect) it's important that other scientists come up with more evidence to validate (confirm) the theory. Other scientists may repeat the study and try to reproduce the results, or conduct other studies to try to prove the same theory.

Example 1: The MMR Vaccine

In 1998, a study was published about the safety of the measles, mumps and rubella (MMR) vaccine. The study was based on 12 children with autism (a life-long developmental disability) and concluded that there may be a link between the MMR vaccine and autism.

Not everyone was convinced by this study because it had a very small sample size of 12 children, which increased the likelihood of the results being due to chance. The study may have been biased because one of the scientists was helping to gain evidence for a lawsuit against the MMR vaccine manufacturer. Also, studies carried out by different scientists found no link between autism and the MMR vaccine.

There have been further scientific studies to sort out the conflicting evidence. In 2005, a Japanese study was published about the incidence of autism in Yokohama (an area of Japan). They looked at the medical records of 30 000 children born between 1988 and 1996 and counted the number of children that developed autism before the age of seven. The MMR jab was first introduced in Japan in 1989 and was stopped in 1993. During this time the MMR vaccine was administered to children at 12 months old. Figure 1 shows the results of the study.

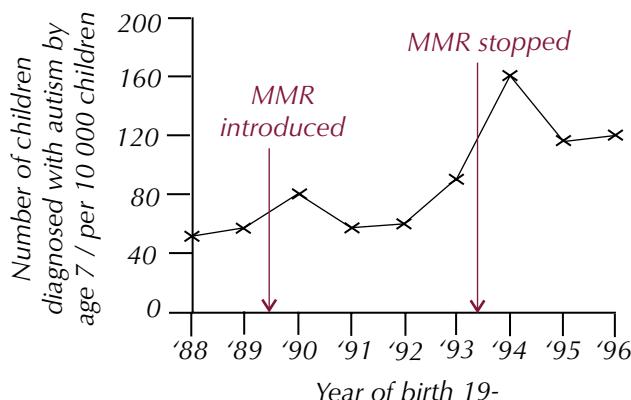


Figure 1: A graph to show the number of children diagnosed with autism by age 7.

In the exam you could be asked to evaluate evidence like this.

You might be asked to describe the data...

The graph shows that the number of children diagnosed with autism continued to rise after the MMR vaccine was stopped. For example, from all the children born in 1992, who did receive the MMR jab, about 60 out of 10 000 were diagnosed with autism before the age of seven. However, from all the children born in 1994, who did not receive the MMR jab, about 160 out of 10 000 of them were diagnosed with autism before the age of seven.

...or draw conclusions

There is no link between the MMR vaccine and autism.

...or evaluate the methodology

You can be much more confident in this study, compared to the 1998 study, because the sample size was so large — 30 000 children were studied.

A larger sample size means that the results are less likely to be due to chance.

Tip: See page 15 for more about drawing conclusions from data and page 17 for more on evaluating methods.

Example 2: Herceptin® — Monoclonal antibodies

About 20% of women with breast cancer have tumours that produce more than the usual amount of a receptor called HER2. Herceptin® is a drug used to treat this type of breast cancer — it contains monoclonal antibodies that bind to the HER2 receptor on a tumour cell and prevent the cells from growing and dividing.

In 2005, a study tested Herceptin® on women who had already undergone chemotherapy for HER2-type breast cancer. 1694 women took the drug for a year after chemotherapy and another 1694 women who were not given the drug were observed for the same time (the control group). The results are shown in Figure 2.

Describe the data: Almost twice as many women in the control group developed breast cancer again or died compared to the group taking Herceptin®.

Draw conclusions:

A one-year treatment with Herceptin®, after chemotherapy, increases the disease-free survival rate for women with HER2-type breast cancer.

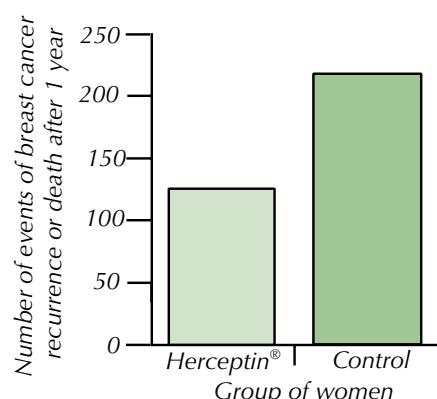


Figure 2: A graph to show the recurrence of breast cancer with and without Herceptin® treatment.

Practice Questions — Application

A vaccination programme was conducted in China in 2009 to protect against influenza type A. A study analysed the side effects of 86.9 million vaccines given between September 2009 and March 2010.

Figure 3 shows the results of the study.

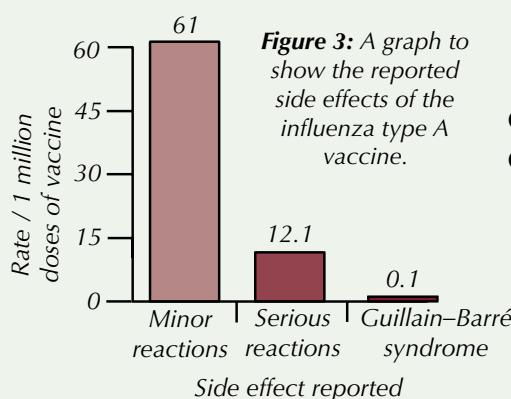


Figure 3: A graph to show the reported side effects of the influenza type A vaccine.

Q1 If 12 million people had the vaccine, how many minor reactions would you expect to see?

Q2 Describe the data.

Q3 The background rate of Guillain-Barré syndrome is 1 per 100 000 people. Does this study support the idea that influenza A vaccination increases the risk of this disease? Explain your answer.

Tip: A background rate is the incidence of something in the general population.

Learning Objectives:

- Understand how the human immunodeficiency virus (HIV) causes the symptoms of AIDS.
- Recall the structure of HIV.
- Recall the stages of HIV replication in helper T-cells.
- Understand why antibiotics are ineffective against viruses.

Specification Reference 3.2.4

Tip: The initial (acute) stage of a HIV infection can cause flu-like symptoms as the immune system mounts a response to the virus. This immune response is not able to destroy all of the virus though, so a small amount remains in the cells and continues to replicate.

Tip: As HIV replicates and the amount of virus increases, the helper T-cell count drops, which leads to AIDS.

Tip: Antiviral drugs (see page 134) can delay the time between HIV and AIDS.

Tip: The infections become more and more serious as there are fewer and fewer immune system cells to fight them.

7. HIV and Viruses

Viruses aren't living things — they can only reproduce inside the cells of another organism. The organism that they infect is called the host.

HIV and AIDS

HIV (**human immunodeficiency virus**) is a virus that affects the human immune system. It eventually leads to **acquired immune deficiency syndrome** (AIDS). AIDS is a condition where the immune system deteriorates and eventually fails. This makes someone with AIDS more vulnerable to other infections, like pneumonia.

HIV host cells

HIV infects and eventually kills **helper T-cells**, which act as the **host cells** (see page 78) for the virus. Helper T-cells send chemical signals that activate phagocytes, cytotoxic T-cells and B-cells (see pages 118-119) so they're hugely important cells in the immune response.

Without enough helper T-cells, the immune system is unable to mount an effective response to infections because other immune system cells don't behave how they should. People infected with HIV develop AIDS when the helper T-cell numbers in their body reach a critically low level.

Initial infection

During the initial infection period, HIV replicates rapidly and the infected person may experience severe flu-like symptoms. After this period, HIV replication drops to a lower level. This is the **latency period**. During the latency period (which can last for years) the infected person won't experience any symptoms.

The symptoms of AIDS

People with HIV are classed as having AIDS when symptoms of their failing immune system start to appear or their helper T-cell count drops below a certain level. The length of time between infection with HIV and the development of AIDS varies between individuals but without treatment it's usually around 10 years. People with AIDS generally develop diseases that wouldn't cause serious problems in people with a healthy immune system.

- The initial symptoms of AIDS include minor infections of mucous membranes (e.g. the inside of the nose, ears and genitals), and recurring respiratory infections.
- As AIDS progresses the number of immune system cells decreases further. Patients become susceptible to more serious infections including chronic diarrhoea, severe bacterial infections and tuberculosis.
- During the late stages of AIDS patients have a very low number of immune system cells and can develop a range of serious infections such as toxoplasmosis of the brain (a parasite infection) and candidiasis of the respiratory system (fungal infection). It's these serious infections that kill AIDS patients, not HIV itself.

The length of time that people survive with AIDS varies a lot. Factors that affect progression of HIV to AIDS and survival time with AIDS include existing infections, the strain of HIV they're infected with, age and access to healthcare.

HIV structure

You might get asked about the structure of HIV in your exam. The basic structure of HIV is shown in Figure 1.

The virus particle has a spherical structure. It's made up of a core containing the genetic material (RNA) and some proteins (including the enzyme **reverse transcriptase**, which is needed for virus replication). It has an outer coating of protein called a capsid and an extra outer layer called an envelope, which is made of membrane stolen from the cell membrane of a previous host cell. Sticking out from the envelope are loads of copies of an attachment protein that help HIV attach to the host helper T-cell.

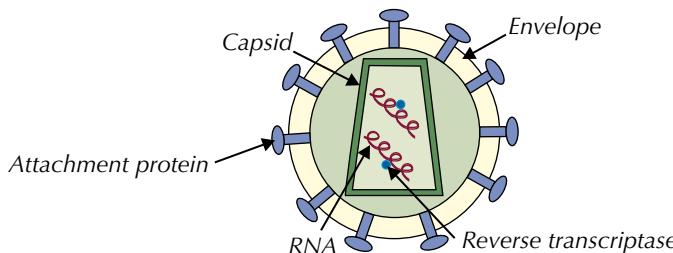


Figure 1: The structure of HIV.

Tip: HIV attachment proteins are foreign antigens that can be recognised by the immune system. During replication, the virus can change parts of the structure of its attachment proteins — this is antigenic variation (see p. 125), and it helps HIV evade destruction by the immune system.

Tip: The attachment proteins are also called envelope proteins.

HIV replication

HIV (and all other viruses) can only reproduce inside the cells of the organism it has infected. HIV replicates inside the helper T-cells of the host. It doesn't have the equipment (such as enzymes and ribosomes) to replicate on its own, so it uses those of the host cell. The following text and Figure 3 show how HIV replicates:

1. The attachment protein attaches to a receptor molecule on the cell membrane of the host helper T-cell.
2. The capsid is released into the cell, where it uncoats and releases the genetic material (RNA) into the cell's cytoplasm.
3. Inside the cell, reverse transcriptase is used to make a complementary strand of DNA from the viral RNA template.
4. From this, double-stranded DNA is made and inserted into the human DNA.
5. Host cell enzymes are used to make viral proteins from the viral DNA found within the human DNA.
6. The viral proteins are assembled into new viruses, which bud from the cell and go on to infect other cells.

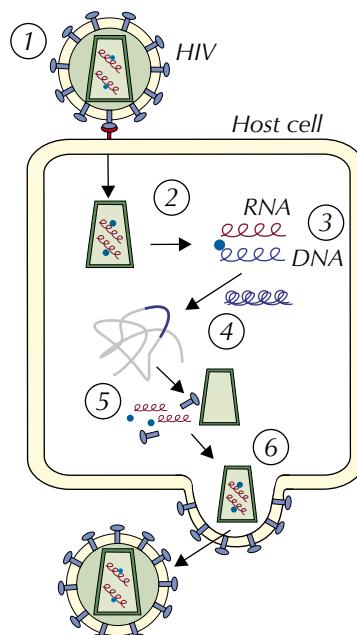


Figure 3: Replication of HIV using a host cell.

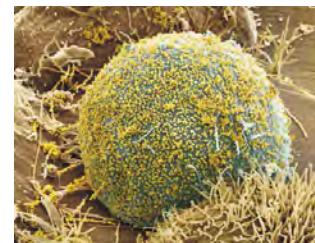


Figure 2: An electron micrograph of a cell (blue sphere) infected by HIV (yellow dots).

Tip: See pages 53-56 for loads more on DNA and RNA and how the two are inter-linked.

Tip: When HIV particles emerge from a cell, the cell ruptures and dies.

Antibiotics and viruses

Tip: Antibiotics don't kill viruses, which is why you don't get prescribed antibiotics for a cold. Colds are caused by rhinoviruses.

Tip: HIV is a type of virus called a retrovirus. So HIV antiviral therapy is also known as antiretroviral therapy.

Tip: HIV testing, based on HIV antibody detection, before a baby is 18 months old can be inaccurate. This is because the baby of an HIV-positive mother may have some HIV antibodies in their blood (passed over from their mother in the womb) regardless of whether or not they're infected.

Antibiotics kill bacteria by interfering with their metabolic reactions. They target the bacterial enzymes and ribosomes used in these reactions. Bacterial enzymes and ribosomes are different from human enzymes and ribosomes. Antibiotics are designed to only target the bacterial ones so they don't damage human cells. Makes sense.

Viruses don't have their own enzymes and ribosomes — they use the ones in the host's cells. So because human viruses use human enzymes and ribosomes to replicate, antibiotics can't inhibit them because they don't target human processes. Most **antiviral drugs** are designed to target the few virus-specific enzymes (enzymes that only the virus uses) that exist.

Example

HIV uses reverse transcriptase to replicate (see previous page). Human cells don't use this enzyme, so drugs can be designed to inhibit it without affecting the host cell. These drugs are called reverse-transcriptase inhibitors.

Controlling HIV infection

There's currently no cure or vaccine for HIV but antiviral drugs can be used to slow down the progression of HIV infection and AIDS in an infected person.

The best way to control HIV infection in a population is by reducing its spread. HIV can be spread via unprotected sexual intercourse, through infected bodily fluids (e.g. blood from sharing contaminated needles) and from a HIV-positive mother to her fetus. Not all babies from HIV-positive mothers are born infected with HIV and taking antiviral drugs during pregnancy can reduce the chance of the baby being HIV-positive.

Practice Questions — Application

Q1 Figure 4 shows the estimated number of deaths from AIDS, number of people diagnosed with AIDS and number of people living with HIV infection aged ≥ 13 years in the United States between the years 1981 and 2008.

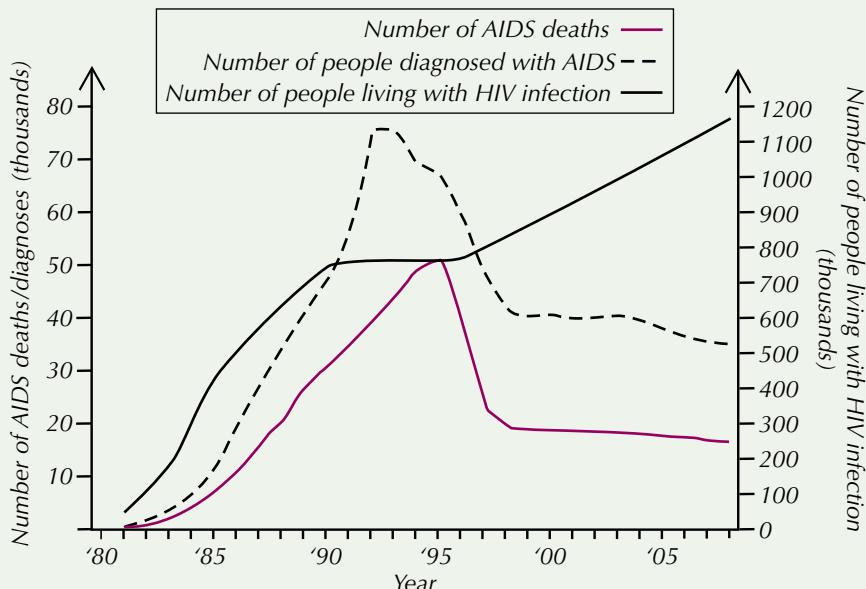


Figure 4: HIV and AIDS statistics from the US between 1981 and 2008

Highly active antiretroviral therapy (HAART) is a combination of several antiretroviral drugs that reduce the amount of HIV in the body.

- Use information from Figure 4 to suggest the year that HAART was first introduced. Give two reasons for your answer.
- Calculate the percentage decrease in the number of AIDS deaths between 1995 and 1998.
- Suggest and explain the effect HAART has on the progression of HIV to AIDS.

Q2 Initial infection with HIV stimulates an immune response, producing antibodies specific to HIV. However, the immune system does not completely destroy the virus.

- Suggest why the immune response is not sufficient to destroy the virus.
- HIV can vary the structure of its attachment proteins. Suggest how this helps the virus evade the immune response when replicating.

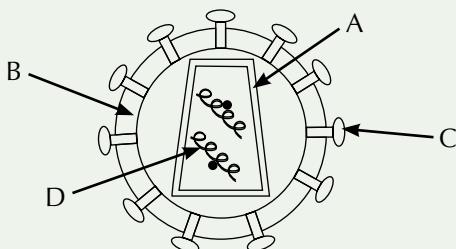
Tip: See page 6 for info on how to calculate percentage decreases.

Practice Questions — Fact Recall

Q1 What does HIV stand for?

Q2 What disease does HIV cause?

Q3 Look at the diagram of a HIV particle below:



Name the structures labelled A-D.

Q4 Describe how HIV replicates.

Q5 Why can't antibiotics be used against viruses?

Section Summary

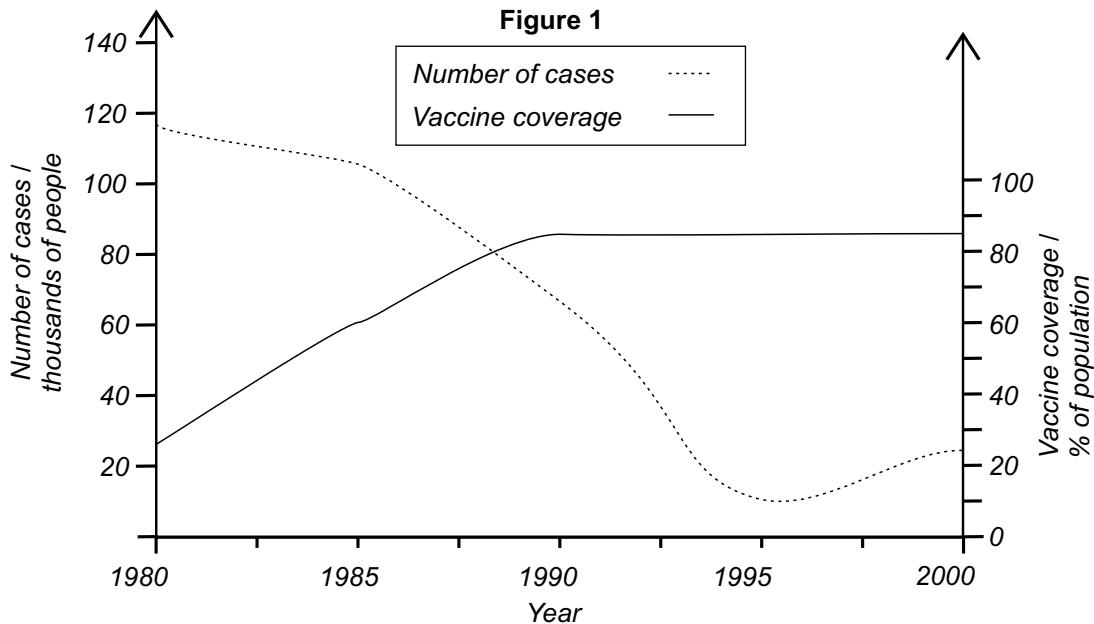
Make sure you know...

- That antigens are molecules (usually proteins) that can generate an immune response.
- That each type of cell has specific antigens on its surface that are used to identify it.
- That the immune system uses antigens to identify foreign cells (e.g. pathogens and cells from another individual), abnormal cells (e.g. cancer cells) and toxins.
- The four main stages of the immune response — phagocytosis, T-cell activation, B-cell activation, and plasma cell and antibody production.
- How phagocytes engulf pathogens, destroy them (using lysozymes) and present their antigens to T-cells.

- That T-cells are activated by foreign antigens presented by phagocytes.
- The helper T-cell response to a foreign antigen — activation of phagocytes, cytotoxic (killer) T-cells and B-cells (clonal selection).
- The B-cell response to a foreign antigen — including division into identical plasma cells and the production and release of monoclonal antibodies specific to the antigen.
- That an antibody is a protein that binds antigens to form an antigen-antibody complex.
- That the formation of antigen-antibody complexes leads to the agglutination and phagocytosis of pathogens.
- The structure of antibodies — including the variable regions (with a unique tertiary structure for antigen binding) and constant regions.
- What the cellular immune response (T-cells) and the humoral immune response (B-cells) are.
- That the primary immune response involves the production of memory cells in response to an antigen.
- That if the same antigen enters the body again, these memory cells will be activated and some will divide to produce plasma cells that produce the right type of antibody to this antigen — and that this is the secondary immune response.
- The differences between active immunity (when your immune system makes its own antibodies) and passive immunity (when you receive antibodies from a different organism).
- How vaccines make people immune to disease by stimulating memory cell production.
- How vaccines protect populations by herd immunity.
- The ethical issues surrounding vaccine use.
- That antigenic variation is when pathogens change their surface antigens.
- The effect antigenic variation has on immunity and disease prevention, e.g. vaccination programmes.
- That monoclonal antibodies are identical antibodies produced from a single group of plasma cells.
- How antibodies can be used in medicine to target specific cell types (e.g. cancer cells) and in medical diagnosis.
- How enzyme-linked antibodies are used in ELISA testing to detect antibodies or antigens of interest for medical diagnosis.
- The ethical issues surrounding the use of monoclonal antibodies.
- How to evaluate methodology, evidence and data relating to the use of vaccines and monoclonal antibodies.
- That human immunodeficiency virus (HIV) causes the symptoms of AIDS by reducing the number of helper T-cells in the body.
- The structure of the HIV and how it replicates in helper T-cells.
- That antibiotics are ineffective against viruses because viruses use host enzymes and ribosomes (so can't be targeted by antibiotics).

Exam-style Questions

- 1 Tetanus is a disease caused by the bacterium *Clostridium tetani*. Symptoms of the disease include extreme muscle spasms caused by the release of a toxin by the bacterium. **Figure 1** shows the global incidence of tetanus along with the percentage vaccine coverage.



- 1.1 Calculate the average rate at which the number of tetanus cases decreased between 1985 and 1990. Give your answer in cases / thousands of people year⁻¹. (2 marks)
- 1.2 In 2011 a newspaper used this data to conclude that, 'Cases of tetanus in the UK are on the increase'. Does the evidence support this conclusion? Explain your answer. (2 marks)
- 1.3 Suggest a possible reason for the increase in cases from 1995 to 2000. (1 mark)
- 1.4 If someone has been potentially exposed to *Clostridium tetani* then they are given a post-exposure injection of antibodies against the toxin. Explain how this prevents them suffering from the disease, but does not prevent them from contracting the disease in the future. (2 marks)
- 2 Phagocytosis is the first stage of the immune response.
- 2.1 Describe the process of phagocytosis. (4 marks)
- 2.2 Outline the main stages of the immune response after phagocytosis. (5 marks)

- 3** The illegal drug amphetamine can be tested for using monoclonal antibodies. Antibodies that bind to amphetamine are created in the laboratory and used to test urine samples.

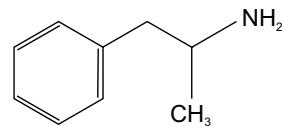
- 3.1** State what monoclonal antibodies are and explain why they are specific to one substance. (3 marks)

- 3.2** Describe the structure of an antibody. (4 marks)

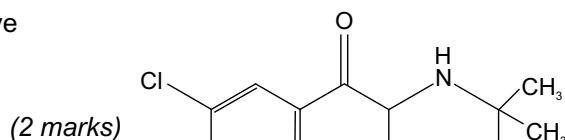
- 3.3** **Figure 2** shows the structures of amphetamine and the prescription drug bupropion.

Taking bupropion can cause a positive result on an amphetamine drug test. Suggest why this happens.

Figure 2



Amphetamine



Bupropion

- 3.4** Describe **one** ethical issue surrounding the use of monoclonal antibodies.

(1 mark)

- 4** Read the following passage:

In 1918 there was a worldwide outbreak of influenza called 'Spanish flu'. It killed approximately 50-100 million people. The virus responsible was the H1N1 strain of influenza — it had type 1 haemagglutinin and type 1 neuraminidase antigens on its surface. Spanish flu circulated the globe for over a year. Survivors of the Spanish flu did not contract the disease when exposed for a second time. 5

The outbreak of Spanish flu killed an unusually high number of young, healthy adults. Some scientists think that in these cases, the patients' helper T-cells produced an excess of chemical signalling molecules. The effect this had on the patients' immune systems may have eventually contributed to their deaths.

In 1957 there was another outbreak of influenza called 'Asian flu'. 10

This outbreak was caused by the H2N2 strain of influenza.

Use the information above and your own scientific knowledge to answer these questions:

- 4.1** Explain why survivors of the Spanish flu did not contract it when exposed for a second time (lines 4-5).

(3 marks)

- 4.2** What effect would an excess of chemical signalling molecules have had on the Spanish flu patients' immune systems (lines 7-9)? Explain your answer.

(2 marks)

- 4.3** Suggest why children and the elderly may not have been affected by Spanish flu in the same way as young adults.

(2 marks)

- 4.4** Survivors of the Spanish flu may have been able to contract Asian flu (line 10). Explain why.

(3 marks)

Topic 3 A: Exchange and Transport Systems

1. Size and Surface Area

Every organism has substances it needs to take in and others it needs to get rid of in order to survive. An organism's size and surface area affect how quickly this is done.

Exchange of substances with the environment

Every organism, whatever its size, needs to exchange things with its environment. Cells need to take in oxygen (for aerobic respiration) and nutrients. They also need to excrete waste products like carbon dioxide and urea. Most organisms need to stay at roughly the same temperature, so heat needs to be exchanged too.

Surface area : volume ratio

An organism's surface area : volume ratio affects how quickly substances are exchanged. But before going into the effects of surface area : volume ratios, you need to understand a bit more about them. Smaller organisms have higher surface area : volume ratios than larger organisms, as shown in the example below.

Example — Maths Skills

A mouse has a bigger surface area relative to its volume than a hippo. This can be hard to imagine, but you can prove it mathematically.

Imagine these animals as cubes...

The mouse could be represented by a cube measuring $1\text{ cm} \times 1\text{ cm} \times 1\text{ cm}$.

Its volume is: $1 \times 1 \times 1 = 1\text{ cm}^3$

Its surface area is: $6 \times 1 \times 1 = 6\text{ cm}^2$

So the mouse has a surface area : volume ratio of **6:1**.

Compare this to a cube hippo measuring $2\text{ cm} \times 4\text{ cm} \times 4\text{ cm}$.

Its volume is: $2 \times 4 \times 4 = 32\text{ cm}^3$

Its surface area is:

$2 \times 4 \times 4 = 32\text{ cm}^2$

(top and bottom surfaces of cube)

$+ (4 \times 2 \times 4) = 32\text{ cm}^2$

(four sides of the cube)

Total surface area = 64 cm^2

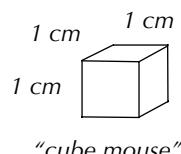
So the hippo has a surface area : volume ratio of $64:32$ or **2:1**.

The cube mouse's surface area is six times its volume, but the cube hippo's surface area is only twice its volume. Smaller animals have a bigger surface area compared to their volume.

Learning Objectives:

- Understand the relationship between the size of an organism or structure and its surface area to volume ratio.
- Know how adaptations, such as changes to body shape and the development of systems in larger organisms, facilitate exchange as the surface area to volume ratio reduces.
- Understand the relationship between surface area to volume ratio and metabolic rate.

Specification Reference 3.3.1



Tip: A ratio shows how big one value is in relation to another.

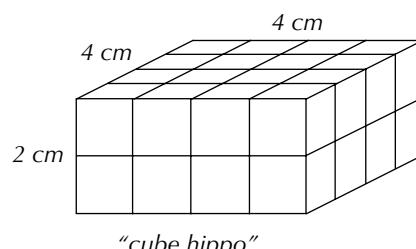
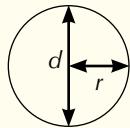


Figure 1: A hippo (top) has a small surface area : volume ratio. A mouse (bottom) has a large surface area : volume ratio.

Exam Tip

It's a good idea to learn the formulae for the area and circumference of a circle as well as for the surface areas and volumes of common 3D shapes, e.g. cubes, cuboids, cylinders and spheres, in case you're asked to calculate one of them in the exam.

Tip: The radius (r) of a circle is the distance from any point on the outer edge of the circle to its centre. It's half the diameter (d).



Tip: Remember that volume is given in units cubed (e.g. μm^3) and surface area is given in units squared (μm^2).

Tip: The formula for calculating the surface area of a sphere is $4\pi r^2$.

Tip: To compare two ratios (e.g. 7:2 and 3:1) it's best to get the last figure in each ratio to be 1 (e.g. 7:2 would become 3.5:1). Then you can easily see which ratio is the largest (e.g. 3.5:1 is a bigger ratio than 3:1).

Calculating volume and surface area

You might be asked to calculate volume or surface area in the exam. For example, you could be asked to calculate the volume or surface area of a cell.

Example — Maths Skills

Bacillus are rod-shaped bacteria — as shown in Figure 2.

To calculate the volume of this cell, you need to split the bacterium into parts: the cylindrical centre and the hemispheres on either end.

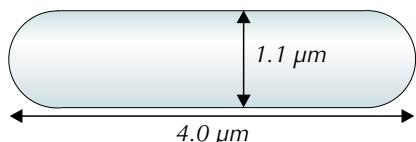
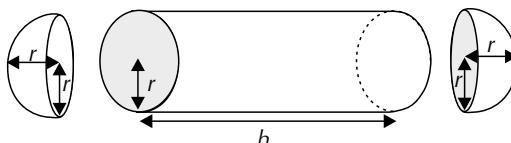


Figure 2: A *Bacillus* cell.



1. Start by calculating the volume of the cylinder. The formula you need is $\pi r^2 h$ or $\pi \times \text{radius}^2 \times \text{height}$. First find the radius, then the height:

$$\begin{aligned}\text{radius } (r) &= 1.1 \div 2 & \text{height } (h) &= 4.0 - 0.55 - 0.55 \\ &= 0.55 \mu\text{m} & &= 2.9 \mu\text{m}\end{aligned}$$

Then use them to calculate the volume of the cylinder:

$$\begin{aligned}\text{Volume of a cylinder} &= \pi r^2 h \\ &= \pi \times 0.55^2 \times 2.9 = 2.755\dots\end{aligned}$$

2. Now find the volume of the two hemispheres.

The formula for the volume of a sphere is $\frac{4}{3} \pi r^3$.

A sphere is made of two hemispheres, so the total volume of the two hemispheres = $\frac{4}{3} \pi \times 0.55^3$

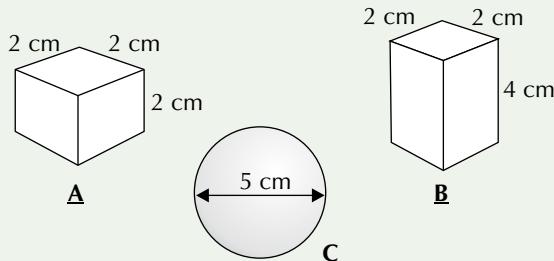
$$= 0.696\dots$$

3. Finally, add the volume of the cylinder and the two hemispheres together to find the total volume of the cell:

$$\begin{aligned}\text{Total volume} &= 2.755\dots + 0.696\dots \\ &= 3.5 \mu\text{m}^3 \text{ (2 s.f.)}\end{aligned}$$

Practice Question — Application

- Q1 Below are three 3D shapes of different sizes (not drawn to scale).



- a) For each 3D shape work out its:
 - i) surface area.
 - ii) volume.
 - iii) surface area:volume ratio.
- b) Which 3D shape has the greatest surface area:volume ratio?

Exchange organs and mass transport systems

An organism needs to supply every one of its cells with substances like glucose and oxygen (for respiration). It also needs to remove waste products from every cell to avoid damaging itself. Different sized organisms do this in different ways:

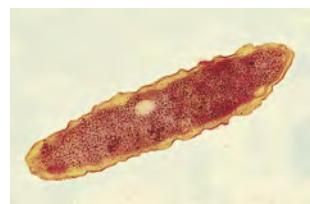


Figure 3: A bacterium — an example of a single-celled organism.

Single-celled organisms

In single-celled organisms, these substances can **diffuse** directly into (or out of) the cell across the cell-surface membrane. The diffusion rate is quick because of the small distances the substances have to travel (see p. 102).

Multicellular organisms

In multicellular organisms, diffusion across the outer membrane is too slow, for two reasons:

1. Some cells are deep within the body — there's a big distance between them and the outside environment.
2. Larger animals have a low surface area to volume ratio — it's difficult to exchange enough substances to supply a large volume of animal through a relatively small outer surface.

So rather than using straightforward diffusion to absorb and excrete substances, multicellular organisms need specialised **exchange organs** (like lungs — see page 148).

They also need an efficient system to carry substances to and from their individual cells — this is **mass transport**. In mammals, 'mass transport' normally refers to the circulatory system, which uses blood to carry glucose and oxygen around the body. It also carries hormones, antibodies and waste like CO₂. Mass transport in plants involves the transport of water and solutes in the xylem and phloem.

Heat exchange

As well as creating waste products that need to be transported away, the metabolic activity inside cells creates heat. Staying at the right temperature is difficult, and it's pretty heavily influenced by your size and shape...

Body size

The rate of heat loss from an organism depends on its surface area. As you saw on page 139, if an organism has a large volume, e.g. a hippo, its surface area is relatively small. This makes it harder for it to lose heat from its body. If an organism is small, e.g. a mouse, its relative surface area is large, so heat is lost more easily. This means smaller organisms need a relatively high metabolic rate, in order to generate enough heat to stay warm.

Body shape

Animals (of any size) with a compact shape have a small surface area relative to their volume — minimising heat loss from their surface. Animals with a less compact shape (those that are a bit gangly or have sticky outy bits) have a larger surface area relative to their volume — this increases heat loss from their surface.

Adaptations for heat exchange

Whether an animal is compact or not depends on the temperature of its environment — the animal's body shape is **adapted** to suit its environment.

Tip: Remember, diffusion is the net movement of particles from an area of higher concentration to an area of lower concentration — see page 102.

Tip: There's more about the circulatory system on page 175. There's more about xylem and phloem on pages 191 and 195.

Examples

Arctic fox

Body temperature — 37 °C
Average outside temperature — 0 °C



The Arctic fox has small ears and a round head to reduce its surface area : volume ratio and heat loss.

African bat-eared fox

Body temperature — 37 °C
Average outside temperature — 25 °C



The African bat-eared fox has large ears and a more pointed nose to increase its surface area : volume ratio and heat loss.

European fox

Body temperature — 37 °C
Average outside temperature — 12 °C



The European fox is intermediate between the two, matching the temperature of its environment.



Figure 4: A squirrel eats high energy foods to fuel its high metabolic rate.



Figure 5: An elephant's large, flat ears help it keep cool.

Exam Tip

Make sure you write about surface area : volume ratio in the exam and not just surface area.

Behavioural and physiological adaptations to aid exchange

Not all organisms have a body size or shape to suit their climate — some have other adaptations to aid exchange instead...

- Animals with a high surface area : volume ratio tend to lose more water as it evaporates from their surface. This is a problem particularly for animals living in hot regions where water evaporates quickly. Some small desert mammals have kidney structure adaptations so that they produce less urine to compensate.
- To support their high metabolic rates, small mammals living in cold regions need to eat large amounts of high energy foods such as seeds and nuts.
- Smaller mammals may have thick layers of fur or hibernate when the weather gets really cold.
- Larger organisms living in hot regions, such as elephants and hippos, find it hard to keep cool as their heat loss is relatively slow. Elephants have developed large flat ears which increase their surface area, allowing them to lose more heat. Hippos spend much of the day in the water — a behavioural adaptation to help them lose heat.

Practice Questions — Application

Q1 An Emperor penguin is much larger than an Adélie penguin.

- Which penguin would you expect to have the larger surface area : volume ratio?
- Which penguin would you expect to find in the coldest regions? Explain your answer.

An Adélie penguin has a compact shape with short wings and legs. A Rockhopper penguin is less compact with longer wings and legs.

- Assuming the two penguins are roughly the same size, explain which one you would expect to live in the colder regions.

- Q2 In snowy, winter months small animals such as mice and voles live in underground tunnels. Suggest why they have developed this behaviour.
- Q3 In winter some birds ‘fluff’ their feathers to trap more warm air close to their body. Would you expect this physiological adaptation to be more common among small or large birds? Explain your answer.
- Q4 Some large desert animals, such as coyotes, sleep during the day and are only active at night. Suggest why they have this behaviour.

Practice Questions — Fact Recall

- Q1 a) Name two substances an animal needs to take in from its environment.
b) Name two substances an animal needs to release into its environment.
- Q2 Do most large animals have a higher or lower surface area:volume ratio than small animals?
- Q3 Give two reasons why diffusion is too slow in multicellular organisms for them to absorb and excrete substances this way.
- Q4 What is meant by a ‘mass transport’ system?
- Q5 Will the rate of heat loss at a given temperature be greater for an animal with a high or low surface area:volume ratio?
- Q6 Explain how an animal’s shape can help to control its temperature.
- Q7 Other than body size or shape, give two adaptations a small animal may have to survive in a cold environment.
- Q8 Other than body size or shape, give two adaptations a large animal might have to survive in a hot environment.

Learning Objectives:

- Know the adaptations of gas exchange surfaces, shown by gas exchange:
 - across the body surface of a single-celled organism,
 - across the gills of fish (gill lamellae and filaments including the counter-current principle),
 - by the leaves of dicotyledonous plants (mesophyll and stomata),
 - in the tracheal system of an insect (tracheae, tracheoles and spiracles).
- Understand the structural and functional compromises between the opposing needs for efficient gas exchange and the limitation of water loss shown by terrestrial insects and xerophytic plants.

Specification Reference 3.3.2

Tip: There's more on factors that increase the rate of diffusion on page 102.

Tip: The gills are located inside a fish's head underneath gill slits or a bony flap called the operculum.



Figure 1: The gills inside a mackerel.

2. Gas Exchange

Organisms are constantly exchanging gases with their environment. In large organisms that's not always easy — so many plants and animals have adaptations to aid gas exchange.

Gas exchange surfaces

Gas exchange occurs over a **gas exchange surface** — a boundary between the outside environment and the internal environment of an organism. Organisms need oxygen and carbon dioxide to diffuse across gas exchange surfaces as quickly as possible. Most gas exchange surfaces have two things in common that increase the rate of diffusion:

1. They have a large surface area.
2. They're thin (often just one layer of epithelial cells) — this provides a short diffusion pathway across the gas exchange surface.

The organism also maintains a steep concentration gradient of gases across the exchange surface, which increases the rate of diffusion.

Gas exchange in single-celled organisms

Single-celled organisms absorb and release gases by diffusion through their cell-surface membranes. They have a relatively large surface area, a thin surface and a short diffusion pathway (oxygen can take part in biochemical reactions as soon as it diffuses into the cell) — so there's no need for a specialised gas exchange system.

Gas exchange in fish

There's a lower concentration of oxygen in water than in air. So fish have special adaptations to get enough of it. In a fish, the gas exchange surface is the gills.

Structure of gills

Water, containing oxygen, enters the fish through its mouth and passes out through the gills. Each gill is made of lots of thin plates called **gill filaments**, which give a large surface area for exchange of gases (and so increase the rate of diffusion). The gill filaments are covered in lots of tiny structures called **lamellae**, which increase the surface area even more — see Figure 2.

The lamellae have lots of blood capillaries and a thin surface layer of cells to speed up diffusion, between the water and the blood.

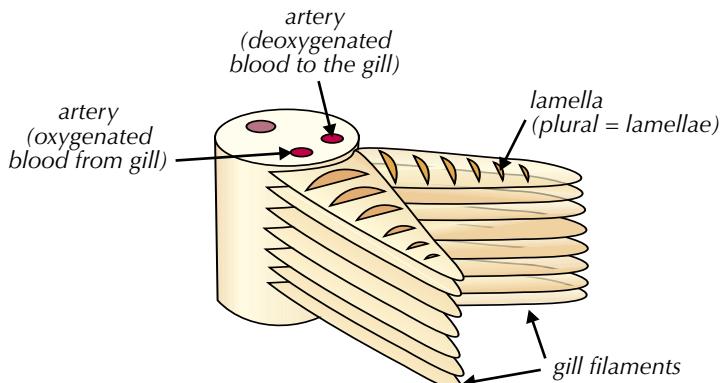


Figure 2: A section of a fish's gill.

The counter-current system

In the gills of a fish, blood flows through the lamellae in one direction and water flows over them in the opposite direction — see Figure 3. This is called a counter-current system. The counter-current system means that the water with a relatively high oxygen concentration always flows next to blood with a lower concentration of oxygen. This in turn means that a steep concentration gradient is maintained between the water and the blood — so as much oxygen as possible diffuses from the water into the blood.

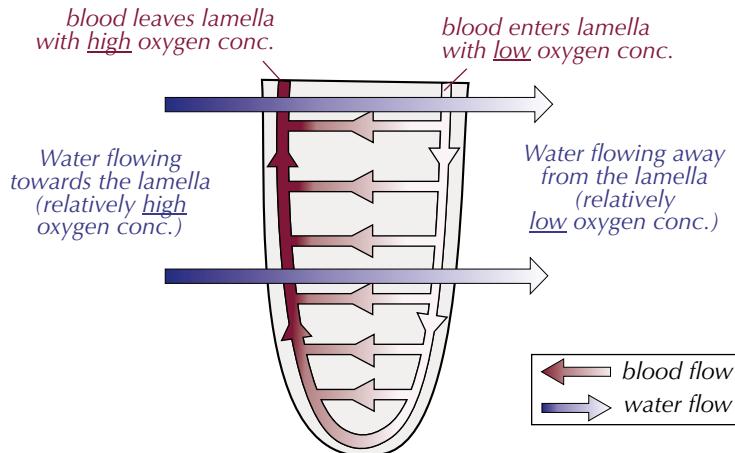


Figure 3: The counter-current system across a lamella.

Exam Tip

In the exam it's not enough to write that the counter-current system creates a steep concentration gradient — you need to say that the concentration gradient is maintained over the whole length of the gill to get the marks.

Tip: The normal circulation of the fish replaces the oxygenated blood that leaves the gill with more deoxygenated blood. The normal ventilation of the fish ensures that more water with a relatively high oxygen concentration is taken in. Both of these help to maintain the steep concentration gradient.

Gas exchange in dicotyledonous plants

Plants need CO₂ for photosynthesis, which produces O₂ as a waste gas. They need O₂ for respiration, which produces CO₂ as a waste gas. The main gas exchange surface is the surface of the **mesophyll cells** in the leaf. They're well adapted for their function — they have a large surface area.

The mesophyll cells are inside the leaf. Gases move in and out through special pores in the epidermis (mostly the lower epidermis) called **stomata** (singular = stoma). The stomata can open to allow exchange of gases, and close if the plant is losing too much water. **Guard cells** control the opening and closing of stomata.

Tip: You don't need to worry too much about what dicotyledonous means — it's a category of plant that includes most green and non-woody plants, bushes and trees.

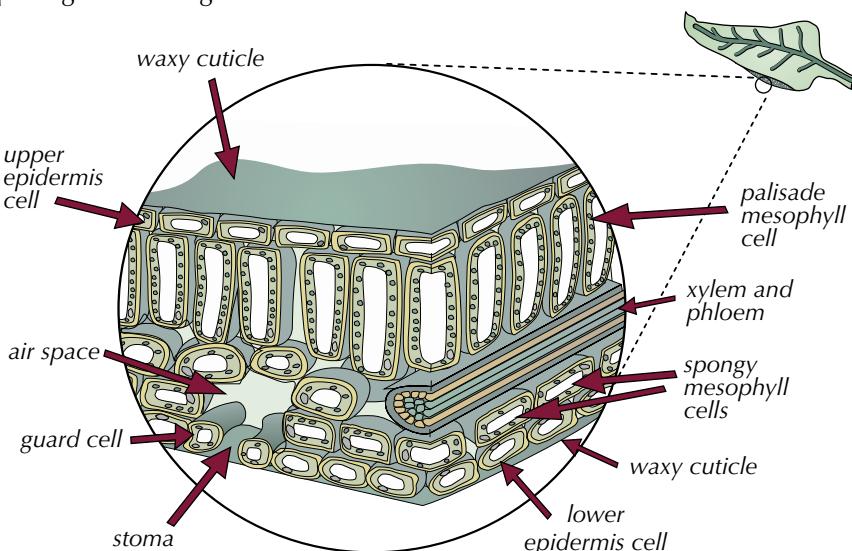


Figure 4: Structure of a dicotyledonous plant leaf.

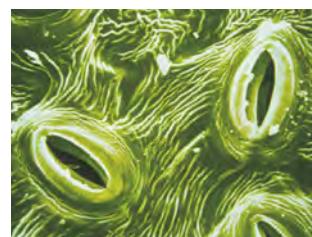


Figure 5: Open stomata on the epidermis of a leaf.

Tip: Terrestrial insects are just insects that live on land.



Figure 6: A spiracle on the surface of a garden tiger moth caterpillar.

Tip: Being turgid means the guard cells become swollen/plump. Being flaccid means they become limp.

Tip: Marram grass and cacti are good examples of xerophytic plants.



Figure 8: Marram grass.

Tip: See page 192 for more on water loss in plants.

Gas exchange in insects

Terrestrial insects have microscopic air-filled pipes called **tracheae** which they use for gas exchange. Air moves into the tracheae through pores on the surface called **spiracles**. Oxygen travels down the concentration gradient towards the cells. The tracheae branch off into smaller **tracheoles** which have thin, permeable walls and go to individual cells. This means that oxygen diffuses directly into the respiring cells — the insect's circulatory system doesn't transport O₂. Carbon dioxide from the cells moves down its own concentration gradient towards the spiracles to be released into the atmosphere. Insects use rhythmic abdominal movements to move air in and out of the spiracles.

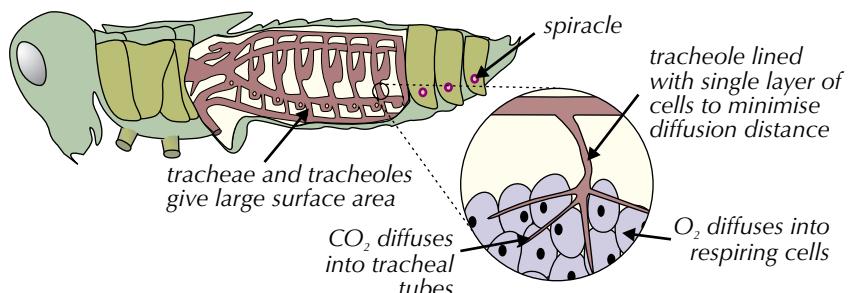


Figure 7: Gas exchange across the tracheal system of an insect.

Control of water loss

Exchanging gases tends to make you lose water — there's a sort of trade-off between the two. Luckily for plants and insects though, they've evolved adaptations to minimise water loss without reducing gas exchange too much.

If insects are losing too much water, they close their spiracles using muscles. They also have a waterproof, waxy cuticle all over their body and tiny hairs around their spiracles, both of which reduce evaporation.

Plants' stomata are usually kept open during the day to allow gaseous exchange. Water enters the guard cells, making them turgid, which opens the stomatal pore. If the plant starts to get dehydrated, the guard cells lose water and become flaccid, which closes the pore.

Some plants are specially adapted for life in warm, dry or windy habitats, where water loss is a problem. These plants are called **xerophytes**. Examples of xerophytic adaptations include:

- Stomata sunk in pits to trap water vapour, reducing the concentration gradient of water between the leaf and the air. This reduces evaporation of water from the leaf.
- A layer of 'hairs' on the epidermis to trap water vapour round the stomata.
- Curled leaves with the stomata inside, protecting them from wind (windy conditions increase the rate of diffusion and evaporation).
- A reduced number of stomata, so there are fewer places for water to escape.
- Thicker waxy, waterproof cuticles on leaves and stems to reduce evaporation.

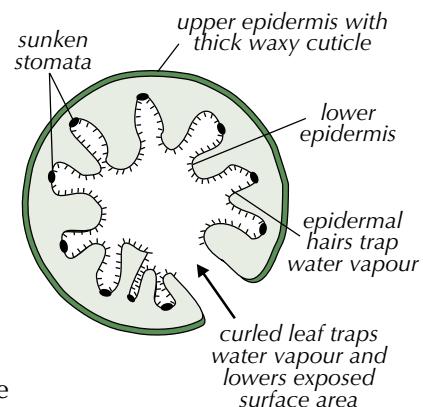
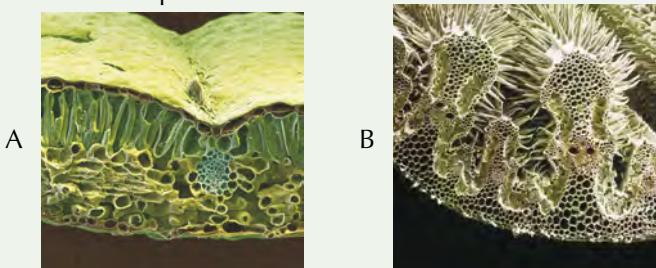


Figure 9: Adaptations of a xerophytic plant.

Practice Questions — Application

- Q1 The photographs below show sections of leaves from two different plants.



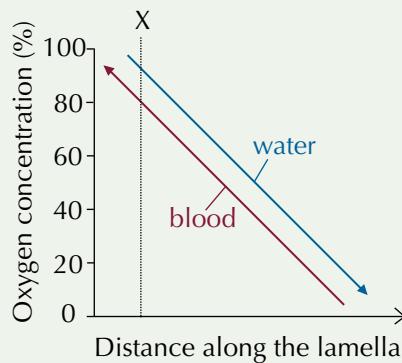
Tip: To help you answer Q1, think of all the adaptations that a xerophytic plant has and then see which photo you can spot them on.

Which leaf belongs to a xerophyte? Explain your answer.

- Q2 In polluted water the dissolved oxygen concentration is lower than it is in clean water. Explain how this would affect gas exchange across the gills of a fish.

- Q3 The graph on the right shows how the relative oxygen concentrations of blood and water change with distance along a lamella.

- What happens to the oxygen concentration of blood as it moves along the lamella?
- What happens to the oxygen concentration of water as it moves along the lamella?
- What is the oxygen concentration of the blood at distance X on the graph?
- Use evidence from the graph to explain why the oxygen concentration of the blood increases straight after point X.



Practice Questions — Fact Recall

- Give two things that all gas exchange surfaces have in common.
- Explain why single-celled organisms don't need a gas exchange system.
- Describe the structure of fish gills.
- Describe how the 'counter-current' system in fish aids gas exchange.
- What is the main gas exchange surface for a dicotyledonous plant?
- Where do gases move in and out of a leaf?
- How does air get into an insect's tracheae?
- Describe how carbon dioxide moves out of an insect's cells into the atmosphere.
- What is a xerophyte?
- Give three adaptations that xerophytic plants have to reduce water loss.

Learning Objectives:

- Know the gross structure of the human gas exchange system including the alveoli, bronchioles, bronchi, trachea and lungs.
- Understand ventilation and the exchange of gases in the lungs.
- Know the mechanism of breathing, including the role of the diaphragm and the antagonistic interaction between external and internal intercostal muscles in bringing about pressure changes in the thoracic cavity.
- Know the essential features of the alveolar epithelium as a surface over which gas exchange takes place.

Specification Reference 3.3.2



Figure 2: A coloured chest X-ray showing the airways in the lungs (pink).

3. Gas Exchange in Humans

The role of your gas exchange system is to supply your blood with oxygen, and remove carbon dioxide from your body.

Gas exchange

Humans need to get oxygen into the blood (for respiration) and they need to get rid of carbon dioxide (made by respiring cells). This is where breathing (or ventilation as it's sometimes called) and the gas exchange system come in.

Structure of the gas exchange system

The structure of the human gas exchange system is shown in Figure 1.

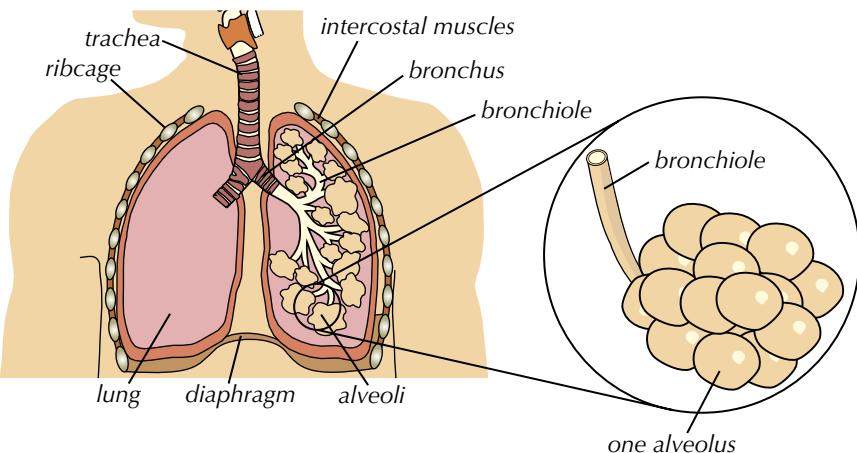


Figure 1: A diagram of the human gas exchange system with the alveoli enlarged.

As you breathe in, air enters the trachea (windpipe). The trachea splits into two bronchi — one bronchus leading to each lung. Each bronchus then branches off into smaller tubes called bronchioles. The bronchioles end in small 'air sacs' called alveoli. This is where gases are exchanged (see next page). The ribcage, intercostal muscles and diaphragm all work together to move air in and out.

Intercostal muscles

The intercostal muscles are found between the ribs. There are actually three layers of intercostal muscles, two of which you need to know about for your exams: the internal and external intercostal muscles. Unsurprisingly, the internal intercostal muscles are on the inside of the external intercostal muscles. There's more about how these two sets of muscles interact to help you breathe on the next page.

Ventilation

Ventilation consists of inspiration (breathing in) and expiration (breathing out). It's controlled by the movements of the diaphragm, internal and external intercostal muscles and ribcage. The processes of inspiration and expiration are described in detail on the next page.

Inpiration

During inspiration the external intercostal and diaphragm muscles contract. This causes the ribcage to move upwards and outwards and the diaphragm to flatten, increasing the volume of the thoracic cavity (the space where the lungs are). As the volume of the thoracic cavity increases, the lung pressure decreases to below atmospheric pressure. Air will always flow from an area of higher pressure to an area of lower pressure (i.e. down a pressure gradient) so air flows down the trachea and into the lungs. Inspiration is an active process — it requires energy.

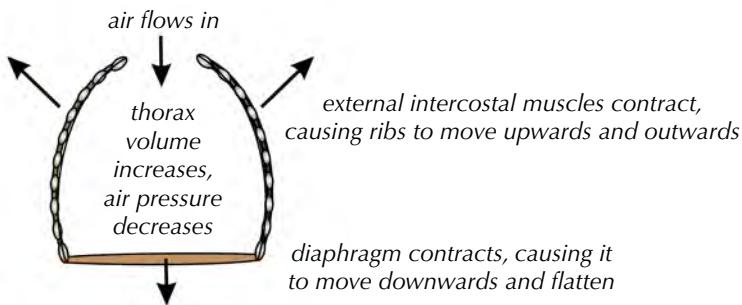


Figure 3: Diagram showing what happens during inspiration.

Tip: Only the external intercostal muscles contract during inspiration — the internal intercostal muscles remain relaxed throughout.

Tip: Remember, when the diaphragm contracts, it's flat. When it relaxes, it bulges upwards. Think of it like trying to hold your stomach in — you contract your muscles to flatten your stomach and relax to release it.

Expiration

During expiration the external intercostal and diaphragm muscles relax. The ribcage moves downwards and inwards, and the diaphragm curves upwards again (so it becomes dome-shaped). The volume of the thoracic cavity decreases, causing the air pressure to increase to above atmospheric pressure. Air is forced down the pressure gradient and out of the lungs.

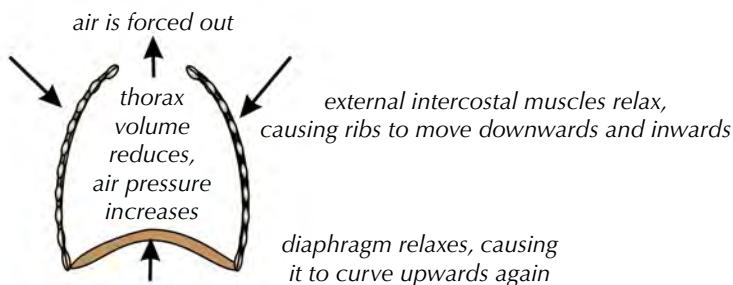


Figure 4: Diagram showing what happens during expiration.

Tip: It's the movement of the ribcage and diaphragm and the change in lung pressure that causes air to flow in and out — not the other way round.

Normal expiration is a passive process — it doesn't require energy. Expiration can be forced though, e.g. if you want to blow out the candles on your birthday cake. During forced expiration, the external intercostal muscles relax and internal intercostal muscles contract, pulling the ribcage further down and in. During this time, the movement of the two sets of intercostal muscles is said to be antagonistic (opposing).

Alveoli and gas exchange

Lungs contain millions of microscopic air sacs where gas exchange occurs — called alveoli. The alveoli are surrounded by a network of capillaries — see Figure 5.

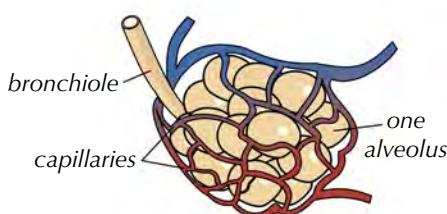


Figure 5: Alveoli covered in a network of capillaries.

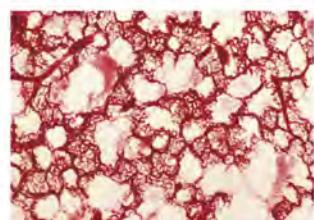


Figure 6: A light micrograph of capillaries surrounding alveoli.

Tip: Epithelial tissue is pretty common in the body. It's usually found on exchange surfaces.

Alveoli structure

The wall of each alveolus is made from a single layer of thin, flat cells called alveolar epithelium. The walls of the capillaries are made from capillary endothelium — see Figure 7.

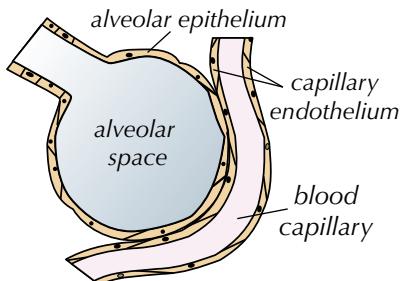


Figure 7: A capillary next to an alveolus.

The walls of the alveoli contain a protein called elastin. Elastin is elastic — it helps the alveoli to return (recoil) to their normal shape after inhaling and exhaling air.

Movement of oxygen and carbon dioxide through the gas exchange system

Air (containing oxygen) moves down the trachea, bronchi and bronchioles into the alveoli. This movement happens down a pressure gradient. Oxygen then moves into the blood where it can be transported round the body — this movement happens down a diffusion gradient.

Carbon dioxide moves down its own diffusion and pressure gradients, but in the opposite direction to oxygen, so that it can be breathed out.

Gas exchange in the alveoli

Tip: Haemoglobin is found in red blood cells. There's more about it on page 170.

Tip: Don't forget, gases pass through two layers of cells (the alveolar epithelium and the capillary endothelium).

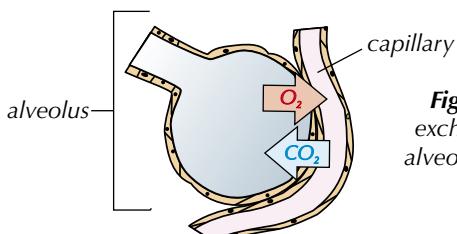


Figure 8: Gaseous exchange between an alveolus and a capillary.

Summary

Figure 9 summarises how oxygen moves through the gas exchange system from when it is first inhaled to reaching the blood. Carbon dioxide moves in the opposite direction.

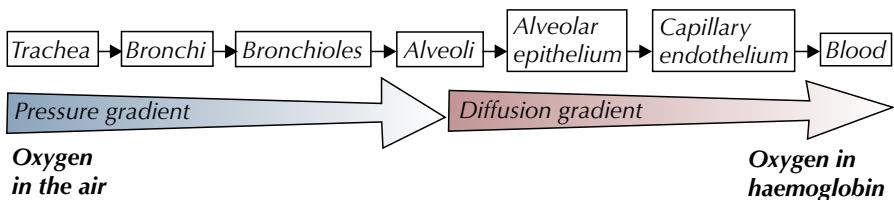


Figure 9: Flow diagram showing how oxygen moves through the gas exchange system.

Factors affecting the rate of diffusion

Alveoli have features that speed up the rate of diffusion so gases can be exchanged quickly:

- A thin exchange surface — the alveolar epithelium is only one cell thick. This means there's a short diffusion pathway (which speeds up diffusion).
- A large surface area — there are millions of alveoli. This means there's a large surface area for gas exchange.

Tip: These features are the same as the features of gas exchange surfaces in other organisms — see pages 144-146.

There's also a steep concentration gradient of oxygen and carbon dioxide between the alveoli and the capillaries, which increases the rate of diffusion. This is constantly maintained by the flow of blood and ventilation (Figure 10).

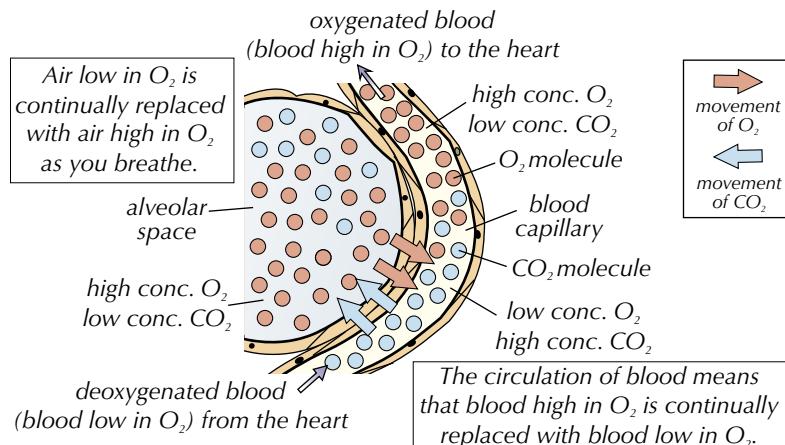


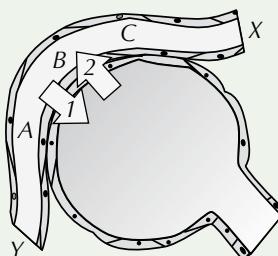
Figure 10: Diagram showing how blood flow and ventilation maintain high concentration gradients of O_2 and CO_2 .

Practice Questions — Application

Q1 The diagram below shows a capillary next to an alveolus.

Blood flows from X to Y.

- Which arrow, 1 or 2, indicates the movement of carbon dioxide?
- At which letter, A, B or C, would you find the highest concentration of oxygen?
- Blood takes 2 s to flow from X to Y. The distance between X and Y is 0.82 mm. Calculate the speed of blood flow from X to Y, giving your answer in mm s^{-1} .



Exam Tip

You need to be able to use maths in the exam — whatever the topic.

Q2 A mountain climber is climbing at altitude, where there's less oxygen. Suggest how this will affect gas exchange in the alveoli.

Practice Questions — Fact Recall

- Describe what happens to make the volume of the thorax increase during inspiration.
- What happens to make air leave the lungs during forced expiration?
- Describe how oxygen gets from the lungs into the blood.
- Describe the features of alveoli and explain how they affect the rate of diffusion.

Learning Objective:

- Be able to interpret information relating to the effects of lung disease on gas exchange and/or ventilation.

Specification Reference 3.3.2

Tip: dm^3 stands for decimetres cubed.
1 dm^3 is the same as 1 litre or 1000 cm^3 .

Exam Tip

Don't be thrown if you get an exam question that uses an unfamiliar measure of lung function. The question will tell you what it means, then it's just a case of applying what you already know.



Figure 1: A coloured X-ray of a patient with pulmonary tuberculosis. The tubercles are shown in pink.

4. The Effects of Lung Disease

Unfortunately, there can be problems with ventilation and gas exchange. You need to be able to interpret information about this.

Lung function

Lung diseases affect both **ventilation** (breathing) and **gas exchange** in the lungs — in other words, how well the lungs function. Here are some terms you might come across in the exams. They're all measures of lung function:

- Tidal volume** is the volume of air in each breath
 - it's usually between 0.4 dm^3 and 0.5 dm^3 for adults.
- Ventilation rate** is the number of breaths per minute. For a healthy person at rest it's about 15 breaths.
- Forced expiratory volume₁ (FEV₁)** is the maximum volume of air that can be breathed out in 1 second.
- Forced vital capacity (FVC)** is the maximum volume of air it is possible to breathe forcefully out of the lungs after a really deep breath in.

Lung diseases

Lung diseases affect lung function in different ways.

Example — Tuberculosis

Pulmonary tuberculosis (TB) is a lung disease caused by bacteria. When someone becomes infected with tuberculosis bacteria, immune system cells build a wall around the bacteria in the lungs. This forms small, hard lumps known as tubercles (see Figure 1). Infected tissue within the tubercles dies and the gaseous exchange surface is damaged, so tidal volume is decreased. TB also causes fibrosis (see below), which further reduces the tidal volume.

A reduced tidal volume means less air can be inhaled with each breath. In order to take in enough oxygen, patients have to breathe faster, i.e. ventilation rate is increased. Common symptoms include a persistent cough, coughing up blood and mucus, chest pains, shortness of breath and fatigue.

Example — Fibrosis

Fibrosis is the formation of scar tissue in the lungs (see Figure 2). This can be the result of an infection or exposure to substances like asbestos or dust. Scar tissue is thicker and less elastic than normal lung tissue. This means that the lungs are less able to expand and so can't hold as much air as normal — tidal volume is reduced, and so is FVC (i.e. a smaller volume of air can be forcefully breathed out). There's a reduction in the rate of gaseous exchange — diffusion is slower across a thicker scarred membrane.

Fibrosis sufferers have a faster ventilation rate than normal — to get enough air into their lungs to oxygenate their blood. Symptoms of fibrosis include shortness of breath, a dry cough, chest pain, fatigue and weakness.

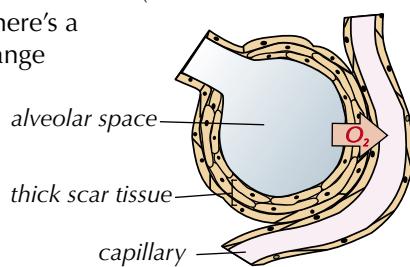


Figure 2: An alveolus with thick scar tissue, which slows the diffusion of O_2 into the capillary.

Example — Asthma

Asthma is a respiratory condition where the airways become inflamed and irritated. The causes vary from case to case but it's usually because of an allergic reaction to substances such as pollen and dust.

During an asthma attack, the smooth muscle lining the bronchioles contracts and a large amount of mucus is produced (see Figure 3). This causes constriction of the airways, making it difficult for the sufferer to breathe properly. Air flow in and out of the lungs is severely reduced, so less oxygen enters the alveoli and moves into the blood. Reduced air flow means that FEV₁ is severely reduced (i.e. less air can be breathed out in 1 second).

Symptoms include wheezing, a tight chest and shortness of breath. During an attack the symptoms come on very suddenly. They can be relieved by drugs (often in inhalers) which cause the muscle in the bronchioles to relax, opening up the airways.

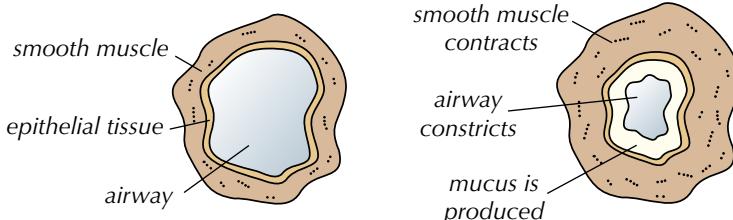


Figure 3: A cross section through a healthy bronchiole (left) and a bronchiole of someone suffering from an asthma attack (right).

Exam Tip

It's a good idea to learn the examples on this page and the previous one for your exams. Although none of these diseases are named in the specification, it's quite likely that if you get an exam question on lung disease, it will be about one of these four.

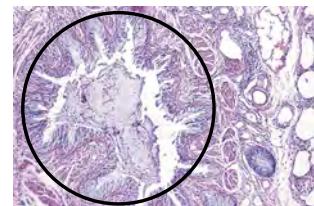


Figure 4: A lung section showing a constricted bronchiole (circled).

Example — Emphysema

Emphysema is a lung disease caused by smoking or long-term exposure to air pollution — foreign particles in the smoke (or air) become trapped in the alveoli. This causes inflammation, which attracts phagocytes to the area. The phagocytes produce an enzyme that breaks down elastin (a protein found in the walls of the alveoli).

Elastin is elastic — it helps the alveoli to return to their normal shape after inhaling and exhaling air. Loss of elastin means the alveoli can't recoil to expel air as well (it remains trapped in the alveoli). It also leads to destruction of the alveoli walls, which reduces the surface area of the alveoli (see Figure 5), so the rate of gaseous exchange decreases.

Symptoms of emphysema include shortness of breath and wheezing. People with emphysema have an increased ventilation rate as they try to increase the amount of air (containing oxygen) reaching their lungs.

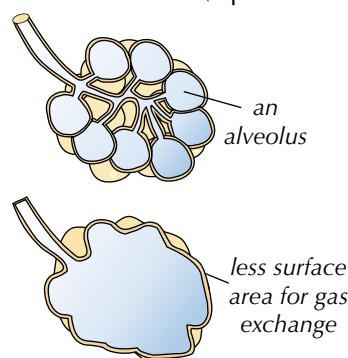


Figure 5: Cross-section of healthy alveoli (top) and damaged alveoli (bottom).

Tip: A phagocyte is a type of white blood cell that carries out phagocytosis — see page 118 for more.

The effect of lung diseases on gas exchange

TB, fibrosis, asthma and emphysema all reduce the rate of gas exchange in the alveoli. Less oxygen is able to diffuse into the bloodstream, the body cells receive less oxygen and the rate of aerobic respiration is reduced. This means less energy is released and sufferers often feel tired and weak.

Interpreting graphs

In the exams, you could be asked to interpret information about lung diseases — and that information includes graphs. Here are two examples of the sort of thing you might get.

Tip: You can also get restrictive lung diseases — these diseases make it difficult to fully breathe in, e.g. fibrosis (in which scar tissue restricts the volume of the lungs).

Exam Tip

You could also be asked to calculate the percentage increase or decrease. Remember that a percentage decrease is always written as a positive number — see page 7.

Tip: If the graph included a line for someone with a restrictive disease, like fibrosis, FVC would be severely reduced because it's hard to get air into the lungs. FEV₁ is likely to be relatively high compared to FVC because someone with a restrictive disease is able to breathe out fairly normally.



Figure 7: Testing a patient's respiratory function using a spirometer.

Example 1 — Maths Skills

Figure 6 shows a typical forced expiratory volume₁ (FEV₁ — the maximum volume of air that can be breathed out in 1 s) and a typical forced vital capacity (FVC — the maximum volume of air it's possible to forcefully breathe out of the lungs) for someone with normal lung function (Line A) and someone with an obstructive lung disease (line B). Obstructive lung diseases are diseases that make it difficult to breathe out, e.g. asthma and emphysema.

You might be asked to do calculations from the data...

For example, you might be asked to calculate the percentage change in FEV₁ for line B compared to line A.

Reading the values off the graph, FEV₁ for line A = 4 dm³ and FEV₁ for line B = 1 dm³.

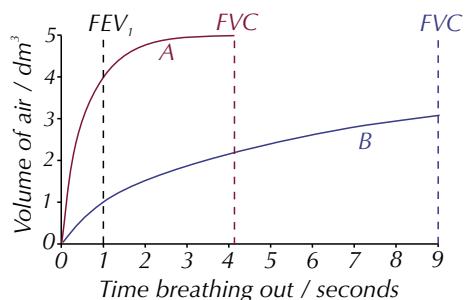


Figure 6: Graph to show typical lung functions.

$$\text{percentage change} = \frac{\text{final value} - \text{original value}}{\text{original value}} \times 100 = \frac{1 - 4}{4} \times 100 = -75\%$$

... and to explain it

FEV₁ and FVC are much lower than normal for someone with an obstructive lung disease because obstructive diseases make it difficult to breathe out. For example, after an asthma attack, the bronchioles are constricted and full of mucus — this narrows the airways, reducing air flow out of the lungs and leading to a large drop in FEV₁. Because it's harder to breathe out, it may also take longer for someone with an obstructive disease to forcibly breathe out all the air in their lungs (i.e. reach their FVC).

Example 2 — Spirometer data

Doctors can carry out tests to investigate lung function and diagnose lung diseases. A machine called a spirometer is used to measure the volume of air breathed in and out. You can figure out tidal volume, ventilation rate and other measures of breathing from the graph produced from a spirometer (see Figure 8).

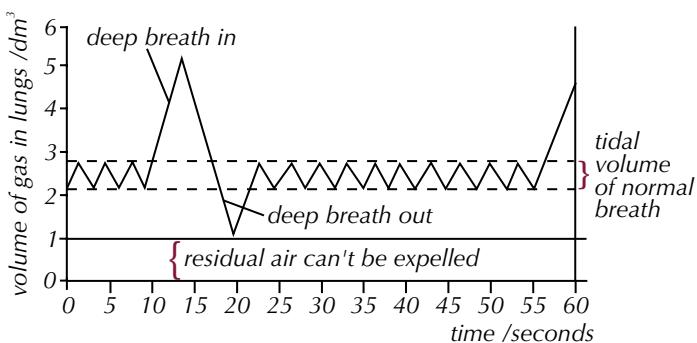
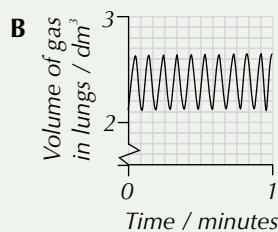
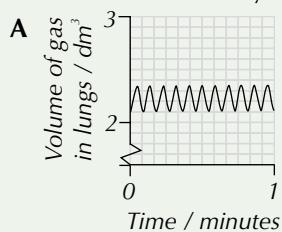


Figure 8: An example of a graph produced by a spirometer.

Practice Questions — Application

- Q1 Which one of the spirometer traces below is from a patient with TB, A or B? Give a reason for your answer.

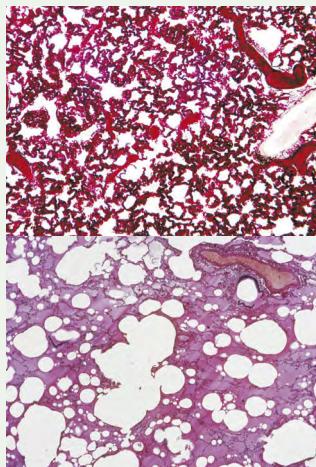


Exam Tip

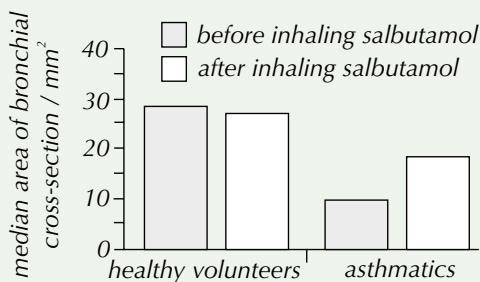
Don't panic if a question asks you about a disease you've never heard of — just apply your knowledge of what you've learnt about how lungs normally work and you'll be able to work out the answer.

- Q2 The pictures on the right show light micrographs of healthy lung tissue (top) and diseased lung tissue from a patient with emphysema (bottom). The alveoli appear white.

- Describe the main difference between the healthy lung tissue and the diseased lung tissue.
- Use your answer to part a) to explain why people with emphysema have a lower level of oxygen in the blood than normal.



- Q3 The graph below shows the median area of a bronchial cross-section in healthy volunteers and in people with asthma. The areas were measured both before and after a drug called salbutamol was inhaled.



- Describe the data.
- Calculate the percentage change in the median bronchial area of asthmatics, after inhaling salbutamol.
- What do you think salbutamol is used for? Explain your answer.

Practice Questions — Fact Recall

- What is forced vital capacity (FVC)?
- A person with fibrosis scar tissue has a reduced tidal volume. Explain why.
- Explain why the rate of gaseous exchange in someone with fibrosis is slower than in a healthy person.
- What happens to FEV_1 during an asthma attack?

Learning Objectives:

- Be able to interpret data relating to the effects of pollution and smoking on the incidence of lung disease.
- Be able to analyse and interpret data associated with specific risk factors and the incidence of lung disease.
- Be able to evaluate the way in which experimental data led to statutory restrictions on the sources of risk factors.
- Be able to recognise correlations and causal relationships.

Specification Reference 3.3.2

Tip: ‘Incidence’ just means the number of new cases of a disease.

Tip: A risk factor is just something that increases the chance of getting a disease. Having a risk factor doesn’t mean you’ll definitely get the disease though, it just makes it more likely.

5. Interpreting Lung Disease Data

It's common for exam questions to include a graph or two, so you could well get asked to interpret some data on the risk factors for lung diseases.

Cause and correlation

All diseases have factors that will increase a person’s chance of getting that disease. These are called **risk factors**. For example, it’s widely known that if you smoke you’re more likely to get lung cancer (smoking is a risk factor for lung cancer). This is an example of a correlation — a link between two things (see page 15). However, a correlation doesn’t always mean that one thing causes the other. Smokers have an increased risk of getting cancer but that doesn’t necessarily mean smoking causes the disease — there are lots of other factors to take into consideration.

Interpreting data

You need to be able to describe and analyse data given to you in your exams. Here’s an example of the sort of thing you might get:

Example 1 — Smoking and lung cancer

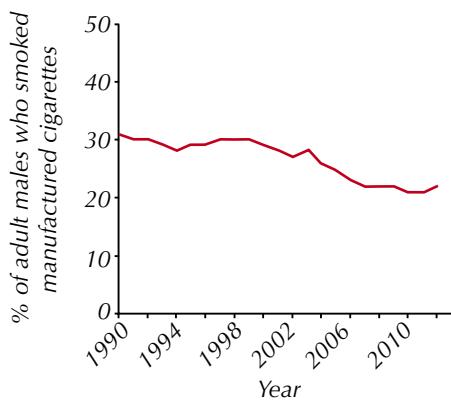


Figure 1: A graph showing the percentage of males aged 16 and over who smoked manufactured cigarettes in Great Britain.

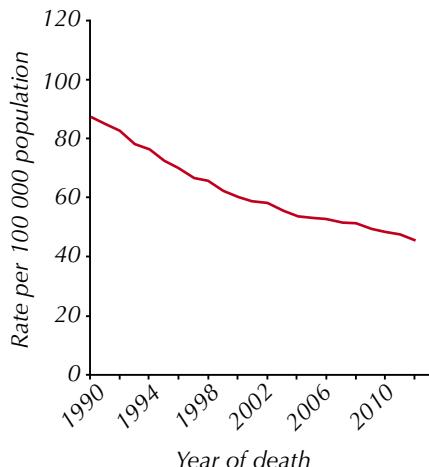


Figure 2: A graph showing age-standardised mortality rates for male lung cancer in the United Kingdom.

You might be asked to describe the data...

Figure 1 shows that the number of adult males in Great Britain who smoke decreased between 1990 and 2012. Figure 2 shows that the male lung cancer mortality (death) rate decreased between 1990 and 2012 in the United Kingdom.

... or draw conclusions

You need to be careful what you say here. There's a correlation (link) between the number of males who smoked and the mortality rate for male lung cancer. But you can't say that one caused the other. There could be other reasons for the trend, e.g. deaths due to lung cancer may have decreased because less asbestos was being used in homes (not because fewer people were smoking).

Other points to consider:

Figure 2 shows mortality (death) rates. The rate of cases of lung cancer may have been increasing but medical advances may mean more people were surviving (so only mortality was decreasing, not the number of people developing the disease).

Exam Tip

It's always a good idea to pick out some numbers from a graph when describing data.

Tip: Mortality rate is the number of deaths in a population in a set period of time (e.g. a year).

Example 2 — Air pollution and asthma

Figure 3 shows the number of new cases of asthma per 100 000 of the population diagnosed in the UK from 1996 to 2000. Figure 4 shows the emissions (in millions of tonnes) of sulfur dioxide (an air pollutant) from 1996 to 2000 in the UK.

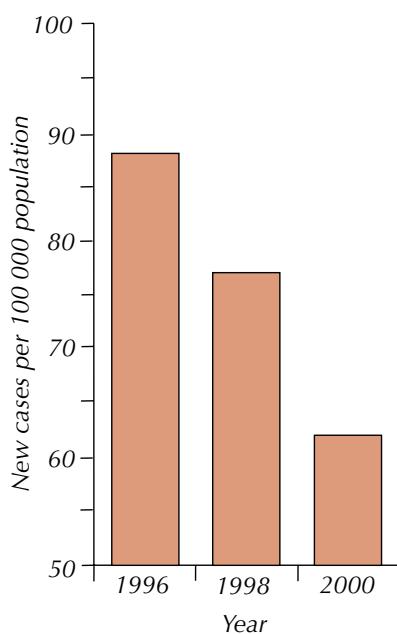


Figure 3: A graph to show the rates of new cases of asthma between 1996 and 2000 in the UK.

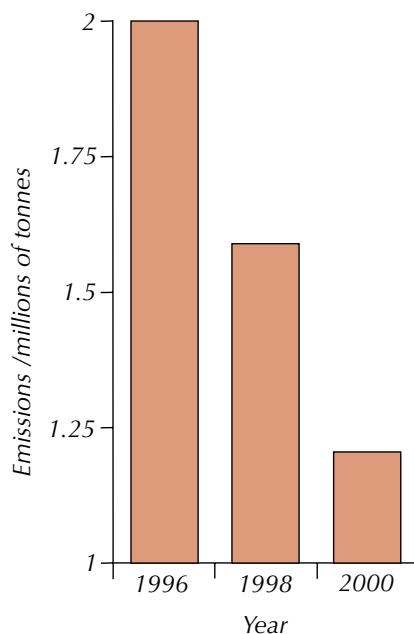


Figure 4: A graph to show the emission of sulfur dioxide between 1996 and 2000 in the UK.

You might be asked to describe the data...

Figure 3 shows that the number of new cases of asthma in the UK fell between 1996 and 2000, from 87 to 62 per 100 000 people.

Figure 4 shows that the emissions of sulfur dioxide in the UK fell between 1996 and 2000, from 2 to 1.2 million tonnes.



Figure 5: Power stations release sulfur dioxide and other pollutants into the atmosphere.

Tip: Figure 4 shows sulfur dioxide emissions in millions of tonnes. So there were 2 million tonnes of emissions in 1996 — not 2 tonnes.

Tip: There's loads more on correlation and cause on page 15.

Tip: Always try to think about other factors that could be affecting results.

... or draw conclusions

Be careful what you say when drawing conclusions. Here there's a link between the number of new cases of asthma and emissions of sulfur dioxide in the UK — the rate of new cases of asthma has fallen as sulfur dioxide emissions have fallen. You can't say that one causes the other though because there could be other reasons for the trend, e.g. the number of new cases of asthma could be falling due to the decrease in the number of people smoking. You can't say the reduction in asthma cases is linked to a reduction in air pollution (in general) either as only sulfur dioxide levels were studied.

Other points to consider:

Figure 3 shows new cases of asthma. The rate of new cases may be decreasing but existing cases may be becoming more severe. The emissions were for the whole of the UK but air pollution varies from area to area, e.g. cities tend to be more polluted. The asthma data doesn't take into account any other factors that may increase the risk of developing asthma, e.g. allergies, smoking, etc.



Figure 6: Cigarette packet showing a written health warning.

Exam Tip

You don't need to learn these examples. They're just here to help you understand how the results of scientific experiments and studies help governments to come up with new laws.

Responses to experimental data

You might also need to evaluate the way in which scientific data has led to government restrictions on the sources of risk factors.

Examples — Restrictions on tobacco and smoking

Advertising of tobacco products

Medical studies in the 1950s and 1960s documented the link between smoking and various forms of cancer, particularly lung cancer. The evidence prompted the first voluntary agreement between the UK government and tobacco companies in 1971, which stated that tobacco products and adverts should carry a health warning label.

However, despite further evidence for smoking-related health risks, it was not until 2003 that bans on advertising of tobacco-based products began to replace the voluntary agreements. As of October 2008, picture health warnings were made compulsory on all UK boxes of cigarettes after studies suggested they were more effective than written warnings alone.

Passive smoking

During the 1980s and 1990s a number of reports were published linking lung-cancer (and other diseases) in non-smokers to smoke that they had been passively exposed to. In 1997, the government initiated a voluntary agreement for workplaces, pubs and restaurants to increase provision of smoke-free areas for non-smokers. However, this had a limited impact.

During the early 2000s, evidence for the effects of passive smoking continued to grow and public support for smoke-free areas increased. In 2002, the British Medical Association called for a ban on smoking in public places. Finally, in 2007, workplaces and public areas such as pubs and restaurants were made smoke-free by law. Increasing concern about the impact of passive smoking on children has recently led to a ban on smoking in cars carrying under-18s, which will come into force in October 2015.

Examples — Restrictions on sources of air pollution

Clean Air Programme for Europe

In response to studies connecting air pollution to various diseases and as part of the Clean Air Programme for Europe, the EU adopted the National Emission Ceilings Directive in 2001. This set upper limits on the total emissions of four major pollutants in the atmosphere, to be achieved by 2010. The four pollutants covered were sulfur dioxides, nitrogen oxides, non-methane volatile organic compounds and ammonia. However, twelve member states failed to meet their emissions targets for at least one pollutant in 2010.

Following a review of progress in 2011 and further scientific evidence, e.g. on the effects of particulate matter on lung function, the directive has been revised. New limits are being agreed on for 2020 with tougher enforcement. Particulate matter has now been included in the emissions targets, along with methane.

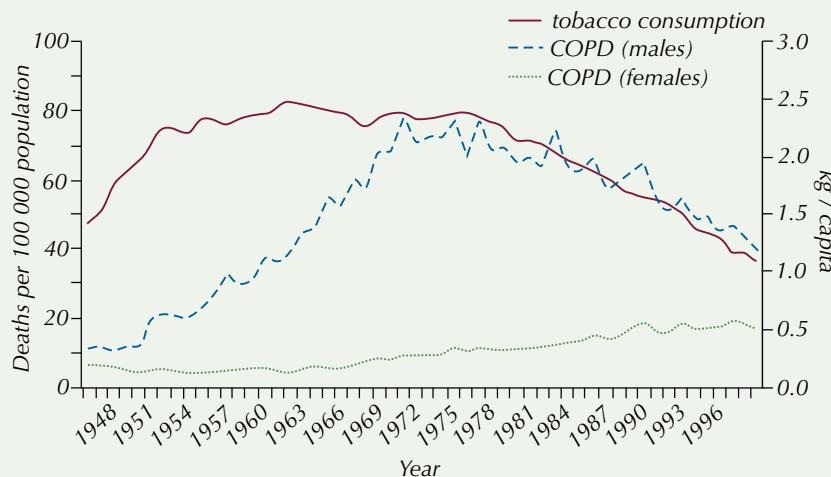
Clean Power for Transport

The EU also introduced the Clean Power for Transport package to promote cleaner fuels for vehicles and all new cars are required to comply with Euro Standards on emissions, which are tested during the car's annual MOT. The UK government also taxes car owners according to their car's emissions with discounts available for cars that have less-polluting fuels.

Tip: The fact that statutory restrictions on sources of air pollution have only been introduced relatively recently is partly because it is difficult to establish air pollution as a cause of lung diseases. This is because there are a number of different pollutants in the atmosphere and it is difficult to monitor people's exposure to them.

Practice Questions — Application

The graph below shows the per capita consumption of tobacco and the death rates for COPD (chronic obstructive pulmonary disease, which includes emphysema) from 1945 to 1998 in Australia.



Tip: Per capita basically just means per person.

- Q1 Describe in detail the trend in male COPD.
- Q2 A scientist concludes from this data that COPD in women is not caused by smoking. Discuss this claim.
- Q3 Suggest how the Australian government might make use of data like this.

Learning Objective:

- Be able to dissect an animal or plant gas exchange system or mass transport system or an organ within such a system (Required Practical 5).

Specification Reference 3.3.4.1

6. Dissecting Gas Exchange Systems

Here's your chance to see what gas exchange systems really look like inside...

Carrying out dissections

REQUIRED PRACTICAL 5

As part of your A-level in Biology, you're expected to carry out at least one dissection. It could be a dissection of a gaseous exchange system or a mass transport system (or an organ within one of those systems) in either an animal or a plant. You could also be asked about dissections in your exams.

There are examples of mass transport system dissections on pages 180 and 193-194. The next two pages cover some gaseous exchange system dissections that you could do as well or instead. Whatever the dissection, you're expected to know how to carry it out safely and ethically. You might also need to record your observations using labelled diagrams.

Dissection tools

Tip: Always follow your teacher's safety instructions when working with dissection tools.



Figure 2: A wax-filled dissection tray.

Tip: You could carry out your dissection on a wooden cutting board instead of in a dissection tray.

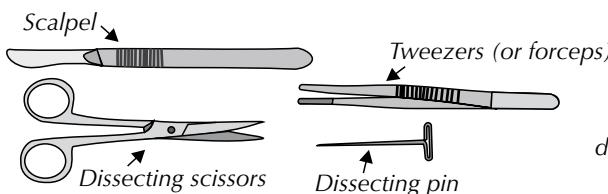


Figure 1: Common dissection tools.

Figure 1 shows some of the tools that you might need to use for your dissections. Scalpels have a very sharp detachable blade and can be used for making very fine cuts. Dissecting scissors are also used for precise cutting. They are safer to use than scalpels (because the blades are less likely to snap under pressure) and it can be easier to avoid damaging the tissue underneath when using scissors. Dissecting pins can be used with a wax-filled dissection tray (see Figure 2) to pin a specimen in place during the dissection. Tweezers are useful for holding and manipulating the smaller parts of the specimen.

Your dissecting tools (e.g. scalpels, dissecting scissors) should all be clean, sharp and free from rust — blunt tools don't cut well and can be dangerous.

Ethical issues

Dissecting animals (including fish and insects) can give you a better understanding of their anatomy. However, there are some ethical issues involved.

Some people argue that it is morally wrong to kill animals just for dissections, as it is unnecessary killing. However many dissections that are carried out in schools involve animals that have already been killed for their meat, e.g. the sheep's lung dissection on the next page. (Some people disagree with killing animals altogether though.)

There are concerns that the animals used for dissections are not always raised in a humane way — they may be subject to overcrowding, extremes of temperature or lack of food — and they may not be killed humanely either. If animals (e.g. insects) are raised in school for dissection, it's important to make sure they are looked after properly and killed humanely to minimise any suffering or distress.

Examples of dissections

REQUIRED
PRACTICAL 5

Example 1 — Dissection of a mammalian lung

1. Lung dissection is messy, so make sure you're wearing a lab coat.
2. Lay the lungs your teacher has given you on a cutting board. They'll probably be sheep or pig lungs from a butcher's shop. You should be able to see the trachea and two bronchi going into the lungs.
3. To see the lungs inflate, pop them in a clear plastic bag, attach a piece of rubber tubing to the trachea and pump air into the lungs using a foot or bicycle pump. The lungs will deflate by themselves because of the elastin in the walls of the alveoli (see p. 150). Never blow down the tube to inflate the lungs — you could end up sucking up stale air from inside the lungs into your mouth. Putting the lungs in a plastic bag stops bacteria inside the lungs from being released into the room.
4. Once you've seen the lungs inflate, you can examine the different tissue types in the lungs.
5. The trachea is supported by C-shaped rings of cartilage.
A cross-section of the trachea is shown in Figure 3.

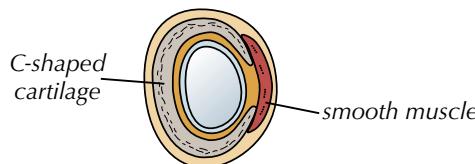


Figure 3: Horizontal cross section through the trachea, showing a C-shaped ring of cartilage.

6. Cartilage is tough, so if you want to open up the trachea, it's best to cut it lengthways, down the gap in the C-shaped rings (see Figure 4). Use dissecting scissors or a scalpel to make the cut. If using a scalpel, cut downwards (not towards you) and don't apply too much pressure to the blade.

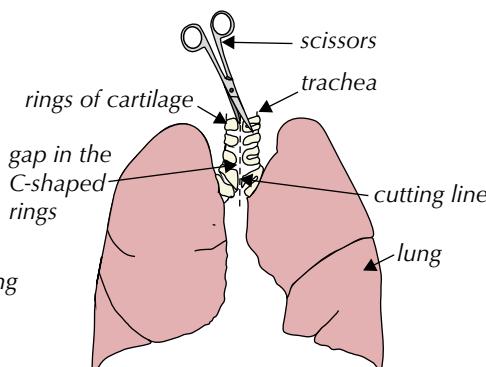


Figure 4: Diagram showing where to cut in order to open up the trachea.

7. Continue cutting down one of the bronchi. You should be able to see the bronchioles branching off.
8. Cut off a piece of the lung. The tissue will feel spongy because of the air trapped in all the alveoli.
9. Lungs from a butcher are safe for humans to handle, but they could still contain bacteria that cause food poisoning. Make sure you wash your hands after the dissection and disinfect work surfaces.

Tip: Make sure that you've done a full risk assessment and identified any safety issues before you start any of these dissections. The risks involved in each one will be slightly different.

Tip: You can learn more about the lungs on pages 148-151.

Tip: The lungs you get from a butcher's will contain cuts from the abattoir, which will allow air to escape when you try to inflate them. If the cuts are making inflation tricky, you could try inflating just one of the lobes (parts) of the lung, which hasn't been cut.

Tip: If you do cut the cartilage be careful — you need to wear goggles to protect your eyes.



Figure 5: Rings of cartilage in the trachea and bronchi.

Example 2 — Dissection of a bony fish

1. Make sure you're wearing an apron or lab coat.
2. Place your chosen fish (something like a perch or salmon works well) in a dissection tray or on a cutting board.

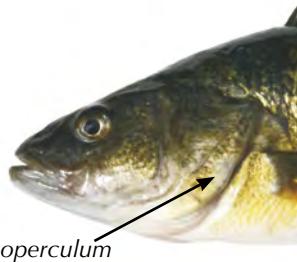


Figure 6: Perch head showing operculum.

Tip: You can find more information about the gas exchange systems of fish on pages 144-145 and insects on page 146.

Tip: Some live insects, e.g. grasshoppers, can cause allergic reactions in some people. They need to be handled very carefully. A full risk assessment should be carried out before using them.

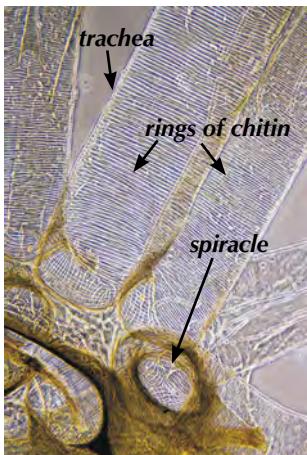


Figure 9: Optical microscope image of a silkworm's shed skin showing the spiracles, chitin rings and silver/grey tracheae.

- Gills are located on either side of the fish's head. They're protected on each side by a bony flap called an operculum and supported by gill arches — see Figure 7.

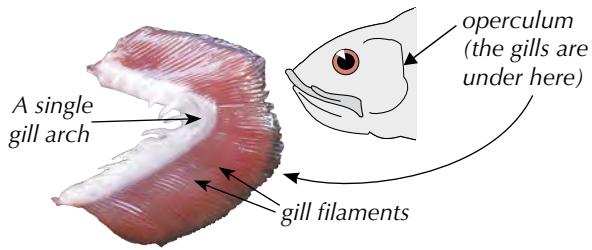


Figure 7: Photo of a fish gill and diagram to show its location beneath operculum.

- To remove the gills, push back the operculum and use scissors to carefully remove the gills. Cut each gill arch through the bone at the top and bottom.
- If you look closely, you should be able to see the gill filaments. With the gills above, it's not possible to see the lamellae without a microscope.

Example 3 — Dissection of a large insect

Big insects like grasshoppers or cockroaches are usually best for dissection because they're easier to handle. For dissection, you'll need to use an insect that's been humanely killed fairly recently.

- First fix the insect to a dissection tray. You can put dissecting pins through its legs to hold it in place.
- To examine the tracheae, you'll need to carefully cut and remove a piece of exoskeleton (the insect's hard outer shell) from along the length of the insect's abdomen — see Figure 8.
- Use a syringe to fill the abdomen with saline solution. You should be able to see a network of very thin, silvery-grey tubes — these are the tracheae. They look silver because they're filled with air.
- You can examine the tracheae under an optical microscope using a temporary mount slide (see p. 83). Again, the tracheae will appear silver or grey. You should also be able to see rings of chitin in the walls of the tracheae — these are there for support (like the rings of cartilage in a human trachea).

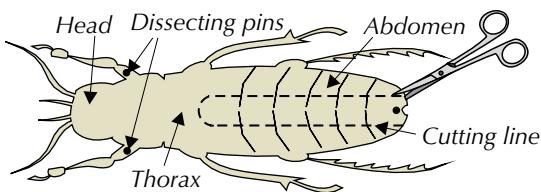


Figure 8: How to remove a piece of exoskeleton from an insect abdomen.

Practice Question — Application

- Q1 A student was dissecting a pair of pig lungs. Her teacher told her to inflate the lungs with a bicycle pump.
- Explain why she shouldn't inflate the lungs by blowing into them.
 - Give one other safety precaution the student should take while inflating the lungs.
 - Once the lungs are inflated, the student stops pumping air into them. Describe and explain what the student would expect to see next.

- d) The student wants to open up the trachea.
Describe where she should make the cut in order to do this.

Practice Questions — Fact Recall

- Q1 How can you make sure that your dissecting tools are safe to use?
Q2 Give two ethical issues that might be raised regarding dissections.

Section Summary

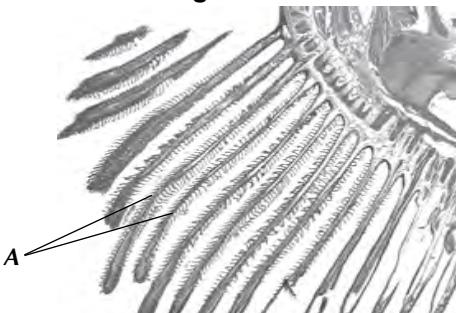
Make sure you know:

- That smaller organisms have a bigger surface area : volume ratio than larger organisms.
- How to calculate the volume and surface area of common 3D shapes.
- That multicellular organisms have adaptations such as specialised exchange organs and mass transport systems to help in the exchange of substances.
- That smaller organisms have a relatively high metabolic rate because their relatively large surface area : volume ratio causes them to lose heat quickly.
- How an animal's body size and shape influence heat exchange.
- That animals have behavioural and physiological adaptations that aid heat exchange.
- How gas exchange surfaces are specialised to increase the rate of diffusion — they're thin and have a large surface area.
- How single-celled organisms are adapted for gas exchange.
- How the structure of fish gills (including gill filaments and lamellae) and the counter-current system maximise gas exchange.
- That gas exchange in dicotyledonous plants takes place in the leaves, and how they are adapted for efficient gas exchange (mesophyll with a large surface area and stomata).
- How the structure of the gas exchange system in insects (including tracheae and spiracles) maximises gas exchange.
- How insects and xerophytic plants are adapted to control water loss.
- The structure of the gas exchange system in humans, including the lungs, trachea, bronchi, bronchioles and alveoli.
- The mechanism of breathing, including the role of the diaphragm and intercostal muscles in changing the pressure in the thoracic cavity.
- How the internal and external intercostal muscles act antagonistically during forced expiration.
- How oxygen and carbon dioxide are exchanged via diffusion gradients in the alveoli.
- How the alveoli are adapted for efficient gas exchange — they provide a thin exchange surface and a large surface area.
- How to interpret information relating to the effects of lung disease on gas exchange or ventilation.
- How to interpret data relating to the effects of pollution and smoking on lung disease and explain what the data shows.
- How to evaluate the way in which experimental data has led to statutory restrictions for smoking and air pollution.
- How to safely and ethically dissect an organism's gas exchange system or an organ within that system — for example, sheep lungs, a bony fish's gills or insect tracheae.

Exam-style Questions

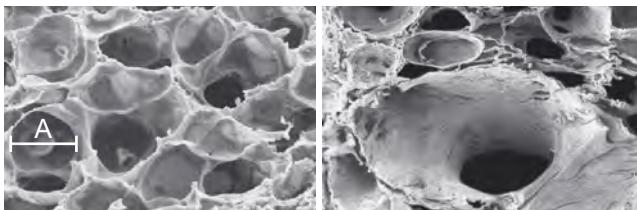
- 1 The gills are the gas exchange organ in fish.
Figure 1 shows a cross section through a dogfish gill.

Figure 1



- 1.1 Name the structures labelled **A** in **Figure 1** and explain how they increase the efficiency of gas exchange across the gills.
(3 marks)
- 1.2 Give **one** other adaptation of the gills for efficient gas exchange.
(1 mark)
- 1.3 Insects have a tracheal system for exchanging gases with the environment. Describe how oxygen gets into an insect's respiring cells.
(2 marks)
- 1.4 Terrestrial insects lose water as a result of gas exchange. Explain **two** features insects have to reduce unwanted water loss.
(2 marks)

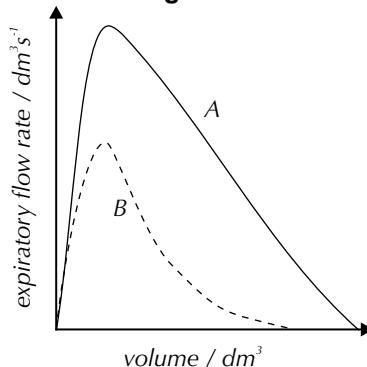
- 2 **Figure 2** shows a scanning electron micrograph of alveoli in a healthy human lung (left) and the effects of emphysema on the alveoli (right). The magnification is x 60.



- 2.1 Calculate the actual width of the labelled alveolus, A. Give your answer in μm .
(2 marks)
- 2.2 Describe **one** difference between the healthy alveoli and the diseased alveoli, and explain what effect this would have on gaseous exchange in the alveoli.
(3 marks)
- 2.3 Oxygen tents contain a higher percentage of oxygen than normal air. Suggest how being in an oxygen tent might benefit a patient with emphysema.
(2 marks)

- 3 Expiratory flow rate is a measure of the volume of air exhaled per second. In a peak expiratory flow test the person inhales as fully as possible and then forcefully exhales all air from the lungs as fast as possible. A doctor measured the expiratory flow rate of two people. The results are shown in **Figure 3**. Line **A** is from a healthy person. Line **B** is from a person shortly after they had an asthma attack. During an asthma attack the bronchioles become inflamed.

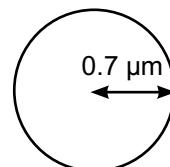
Figure 3



- 3.1 Describe and explain **one** difference between lines **A** and **B** in **Figure 3**. (1 mark)
- 3.2 Some studies have suggested that exercise improves peak expiratory flow rate in healthy adults. Suggest why this might be the case. (2 marks)
- 4 Describe the processes of inspiration and expiration. (6 marks)

- 5 **Figure 4** shows a spherical bacterium with a radius of $0.7 \mu\text{m}$.

Figure 4



- 5.1 Calculate the surface area to volume ratio of this bacterium. (2 marks)
- 5.2 Explain why this bacterium doesn't have a specialised gas exchange system. (3 marks)

Learning Objectives:

- Know that during digestion, large biological molecules are hydrolysed to smaller molecules that can be absorbed across cell membranes.
- Understand the digestion in mammals of:
 - carbohydrates by amylases and membrane bound disaccharidases.
 - lipids by lipase, including the action of bile salts.
 - proteins by endopeptidases, exopeptidases and membrane-bound dipeptidases.
- Know the mechanisms for the absorption of the products of digestion by cells lining the ileum, including:
 - co-transport mechanisms for the absorption of monosaccharides.
 - the role of micelles in the absorption of lipids.
 - co-transport mechanisms for the absorption of amino acids.

Specification Reference 3.3.3

Tip: There's more on polysaccharides, disaccharides and monosaccharides on pages 23-27.

1. Digestion and Absorption

Food molecules need to be broken down by enzymes into smaller molecules. These molecules can then be absorbed into the bloodstream.

Digestion basics

The large biological molecules (e.g. starch, proteins) in food are too big to cross cell membranes. This means they can't be absorbed from the gut into the blood. During digestion, these large molecules are broken down into smaller molecules (e.g. glucose, amino acids), which can move across cell membranes. This means they can be easily absorbed from the gut into the blood, to be transported around the body for use by the body cells.

You might remember from Topic 1A, that most large biological molecules are polymers, which can be broken down into smaller molecules (monomers) using **hydrolysis reactions**. Hydrolysis reactions break bonds by adding water. During hydrolysis, carbohydrates are broken down into disaccharides and then monosaccharides. Fats are broken down into fatty acids and monoglycerides. Proteins are broken down into amino acids.

Digestive enzymes

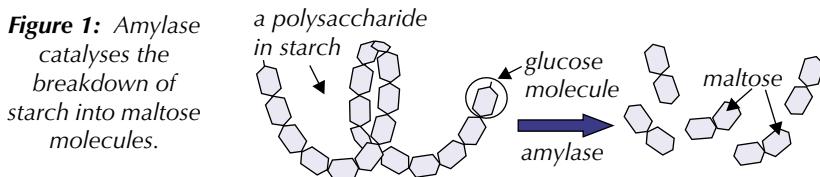
Digestive enzymes are used to break down biological molecules in food. A variety of different digestive enzymes are produced by specialised cells in the digestive systems of mammals. These enzymes are then released to mix with food. Since enzymes only work with specific substrates (see page 39), different enzymes are needed to catalyse the breakdown of different food molecules.

The digestion of carbohydrates

Amylase

Amylase is a digestive enzyme that catalyses the breakdown of starch. Starch is a mixture of two polysaccharides, each made from long chains of alpha-glucose molecules (see page 27). Amylase works by catalysing hydrolysis reactions that break the glycosidic bonds in starch to produce maltose (a disaccharide) — see Figure 1. Amylase is produced by the salivary glands, which release amylase into the mouth, and also by the pancreas, which releases amylase into the small intestine — see Figure 3 (next page).

Figure 1: Amylase catalyses the breakdown of starch into maltose molecules.



Membrane-bound disaccharidases

Membrane-bound disaccharidases are enzymes that are attached to the cell membranes of epithelial cells lining the ileum (the final part of the small intestine). They help to break down disaccharides into monosaccharides. Again, this involves the hydrolysis of glycosidic bonds.

Example

Sucrase is a membrane-bound disaccharidase. It catalyses the breakdown of sucrose into the monosaccharides glucose and fructose.

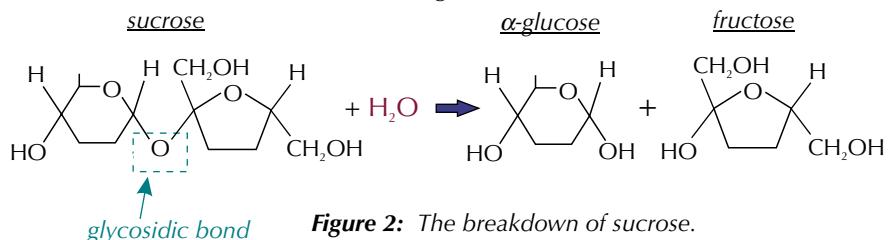


Figure 2: The breakdown of sucrose.

The disaccharides maltose and lactose are broken down in a similar way to sucrose in the example above, i.e. via hydrolysis of the glycosidic bonds. Here's a table showing the enzymes and products involved for all three sugars:

| Disaccharide | Disaccharidase | Monosaccharides |
|--------------|----------------|---------------------|
| sucrose | sucrase | glucose + fructose |
| maltose | maltase | glucose + glucose |
| lactose | lactase | glucose + galactose |

The monosaccharides can be transported across the epithelial cell membranes in the ileum via specific transporter proteins (see page 169).

The digestion of lipids

Lipase enzymes catalyse the breakdown of lipids into monoglycerides and fatty acids. This involves the hydrolysis of the ester bonds in lipids (see Figure 4). Lipases are mainly made in the pancreas — they're then secreted into the small intestine where they act.

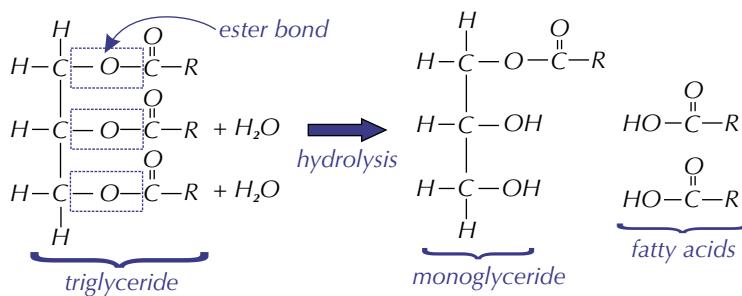


Figure 4: The hydrolysis of ester bonds in a triglyceride.

Bile salts are produced by the liver and **emulsify** lipids — this means they cause the lipids to form small droplets. Although bile salts are not enzymes they are really important in the process of lipid digestion. Several small lipid droplets have a bigger surface area than a single large droplet (for the same volume of lipid). So the formation of small droplets greatly increases the surface area of lipid that's available for lipases to work on.

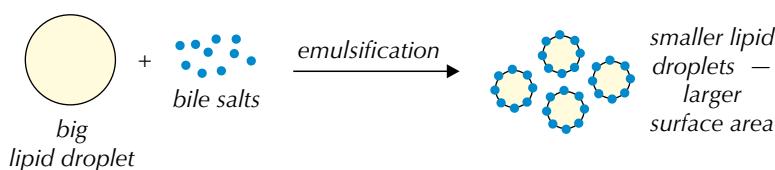


Figure 5: The emulsification of lipids by bile salts.

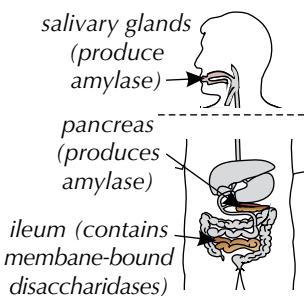


Figure 3: The location of carbohydrate digestive processes in the body.

Tip: The names of most digestive enzymes end with '**ase**'. And you can usually figure out what the enzyme breaks down by looking at what comes before that, e.g. maltase breaks down maltose, lipase breaks down lipids.

Tip: A monoglyceride is a glycerol molecule with one fatty acid attached.

Tip: See pages 29-31 for more information on lipids.

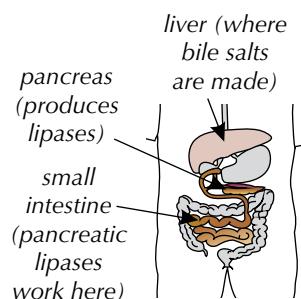


Figure 6: The location of lipid digestive processes in the body.

Once the lipid has been broken down by lipase, the monoglycerides and fatty acids stick with the bile salts to form tiny structures called micelles (see Figure 7). Micelles help the products of lipid digestion to be absorbed (see next page).

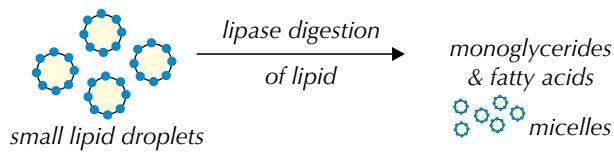


Figure 7: The action of lipase and the formation of micelles.

The digestion of proteins

Tip: Peptidases are also called proteases.

Tip: Remember: endopeptidases break bonds inside the protein.

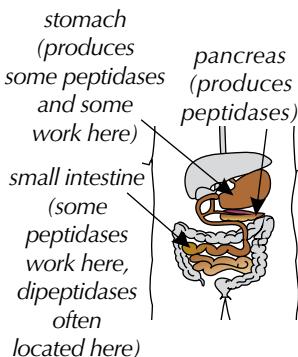


Figure 9: The location of protein digestive processes in the body.

Tip: Dipeptidases located on cell-surface membranes are called 'membrane-bound' dipeptidases.

Proteins are broken down by a combination of different peptidases. These are enzymes that catalyse the conversion of proteins into amino acids by hydrolysing the peptide bonds between amino acids. You need to know about endopeptidases and exopeptidases (including dipeptidases).

Endopeptidases

Endopeptidases act to hydrolyse peptide bonds within a protein (see Figure 8).

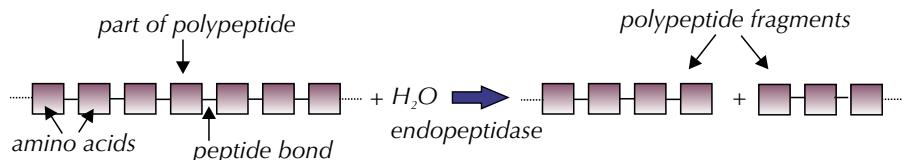


Figure 8: The action of endopeptidases.

Examples

- Trypsin and chymotrypsin are both endopeptidases. They're synthesised in the pancreas and secreted into the small intestine.
- Pepsin is another endopeptidase. It's released into the stomach by cells in the stomach lining. Pepsin only works in acidic conditions — these are provided by hydrochloric acid in the stomach.

Exopeptidases

Exopeptidases act to hydrolyse peptide bonds at the ends of protein molecules. They remove single amino acids from proteins (see Figure 10).

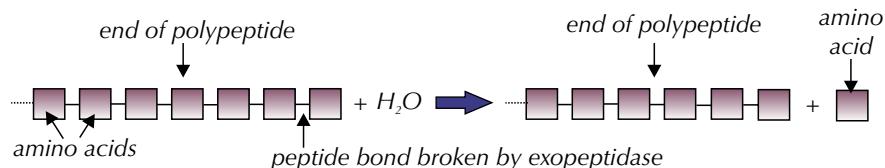


Figure 10: The action of exopeptidases.

Dipeptidases are exopeptidases that work specifically on dipeptides. They act to separate the two amino acids that make up a dipeptide by hydrolysing the peptide bond between them (see Figure 11). Dipeptidases are often located in the cell-surface membrane of epithelial cells in the small intestine.

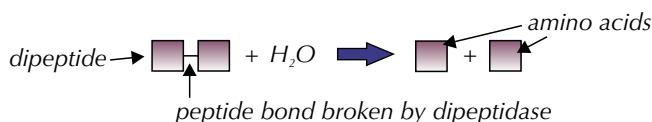


Figure 11: The action of a dipeptidase.

Absorption of the products of digestion

The products of digestion are absorbed across the ileum epithelium into the bloodstream.

Monosaccharides

Glucose is absorbed by active transport with sodium ions via a **co-transporter protein** (see page 111). Galactose is absorbed in the same way using the same co-transporter protein. Fructose is absorbed via facilitated diffusion (see page 103) through a different transporter protein.

Monoglycerides and fatty acids

Micelles (see previous page) help to move monoglycerides and fatty acids towards the epithelium. Because micelles constantly break up and reform they can ‘release’ monoglycerides and fatty acids, allowing them to be absorbed — whole micelles are not taken up across the epithelium. Monoglycerides and fatty acids are lipid-soluble, so can diffuse directly across the epithelial cell membrane.

Amino acids

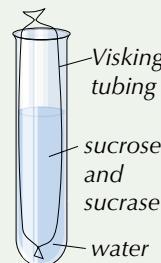
Amino acids are absorbed in a similar way to glucose and galactose. Sodium ions are actively transported out of the epithelial cells into the ileum itself. They then diffuse back into the cells through sodium-dependent transporter proteins in the epithelial cell membranes, carrying the amino acids with them.



Figure 12: Light micrograph of a transverse section through the ileum. The dense network of capillaries (blue/black) helps the products of digestion be absorbed.

Practice Questions — Application

A model gut is set up using Visking tubing as shown in the diagram on the right. Sucrose solution and sucrase are placed inside the Visking tubing and incubated at 37 °C. Sucrose is a disaccharide formed from glucose and fructose. The contents inside and outside the Visking tubing are monitored over time.



- Q1 Sucrase is a membrane-bound disaccharidase. Where in the body would you find sucrase?
- Q2 After 30 minutes, the solution outside the Visking tubing was tested. Name three different molecules that would be present.
- Q3 Explain the role of sucrase in the body.

Tip: Visking tubing is partially permeable — it allows small molecules like water to pass through but not larger molecules like proteins.

Practice Questions — Fact Recall

- Q1 What is a hydrolysis reaction?
- Q2 What type of enzymes are needed to break down lipids?
- Q3 Describe how micelles are formed in digestion.
- Q4 Describe the action of exopeptidases.
- Q5 Explain how monoglycerides and fatty acids are absorbed across the ileum epithelium.
- Q6 How are sodium ions involved in the transport of amino acids?

Learning Objectives:

- Know that the haemoglobins are a group of chemically similar molecules found in many different organisms.
- Know that haemoglobin is a protein with a quaternary structure.
- Understand the role of haemoglobin and red blood cells in the transport of oxygen.
- Understand the loading, transport and unloading of oxygen in relation to the oxyhaemoglobin dissociation curve.
- Understand the cooperative nature of oxygen binding — the change in shape of haemoglobin caused by the binding of the first oxygens makes the binding of further oxygens easier.
- Understand the effects of carbon dioxide concentration on the dissociation of oxyhaemoglobin (the Bohr effect).
- Know that many animals are adapted to their environment by possessing different types of haemoglobin with different oxygen transport properties.

Specification Reference 3.3.4.1

Tip: A protein with a quaternary structure just means it's made up of more than one polypeptide chain — see page 34 for more.

2. Haemoglobin

Many different organisms have haemoglobin in their blood to transport oxygen. But the type of haemoglobin each organism has varies depending on where they live and their way of life...

The role of haemoglobin

Many organisms have to transport substances over large distances to get them to and from their exchange surfaces (see page 141). Mass transport systems, such as the circulatory system in animals, ensure the efficient movement of substances throughout the organism.

Haemoglobin is an important part of the circulatory system. Human haemoglobin is found in red blood cells — its role is to carry oxygen around the body. There are many chemically similar types of haemoglobin found in many different organisms, all of which carry out the same function. As well as being found in all vertebrates, haemoglobin is found in earthworms, starfish, some insects, some plants and even in some bacteria.

Haemoglobin and oxyhaemoglobin

Haemoglobin (Hb) is a large protein with a quaternary structure — it's made up of four polypeptide chains. Each chain has a haem group which contains an iron ion and gives haemoglobin its red colour (see Figure 1). Each molecule of human haemoglobin can carry four oxygen molecules.

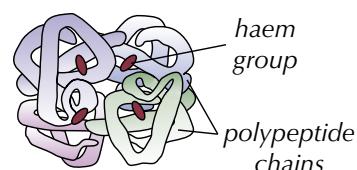


Figure 1: Human haemoglobin.

In the lungs, oxygen joins to haemoglobin in red blood cells to form **oxyhaemoglobin**. This is a reversible reaction — near the body cells, oxygen leaves oxyhaemoglobin and it turns back to haemoglobin (see Figure 2). When an oxygen molecule joins to haemoglobin it's referred to as **association or loading**, and when oxygen leaves oxyhaemoglobin it's referred to as **dissociation or unloading**.

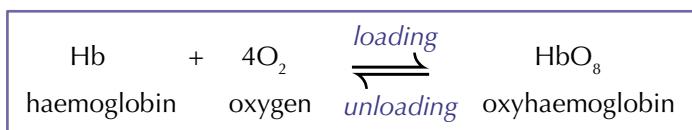


Figure 2: The formation and dissociation of oxyhaemoglobin.

Affinity for oxygen and pO_2

Affinity for oxygen means the tendency a molecule has to bind with oxygen. Haemoglobin's affinity for oxygen varies depending on the conditions it's in — one of the conditions that affects it is the **partial pressure of oxygen (pO_2)**.

pO_2 is a measure of oxygen concentration. The greater the concentration of dissolved oxygen in cells, the higher the partial pressure. As pO_2 increases, haemoglobin's affinity for oxygen also increases:

- Oxygen loads onto haemoglobin to form oxyhaemoglobin where there's a high pO_2 .
- Oxyhaemoglobin unloads its oxygen where there's a lower pO_2 .

Oxygen enters blood capillaries at the alveoli in the lungs. Alveoli have a high pO_2 so oxygen loads onto haemoglobin to form oxyhaemoglobin. When cells respire, they use up oxygen — this lowers the pO_2 . Red blood cells deliver oxyhaemoglobin to respiring tissues, where it unloads its oxygen. The haemoglobin then returns to the lungs to pick up more oxygen. Figure 3 summarises this process.

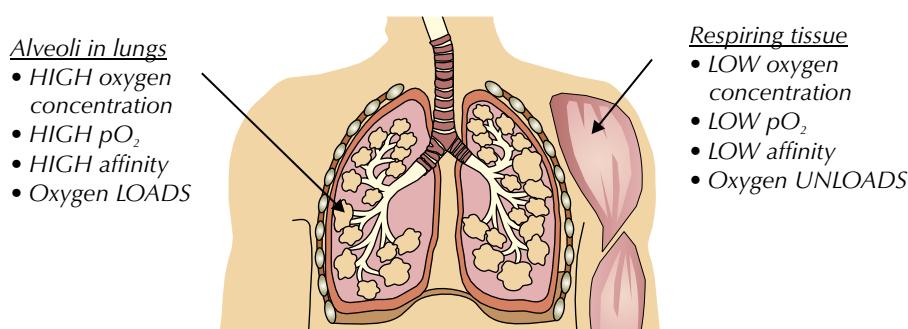


Figure 3: Oxygen loading and unloading in the body.

Exam Tip

Always be specific in your exam answers. For example, don't just say that human haemoglobin has a high affinity for oxygen — it only has a high affinity for oxygen in the lungs.

Dissociation curves

An oxygen dissociation curve shows how saturated the haemoglobin is with oxygen at any given partial pressure. The affinity of haemoglobin for oxygen affects how saturated the haemoglobin is:

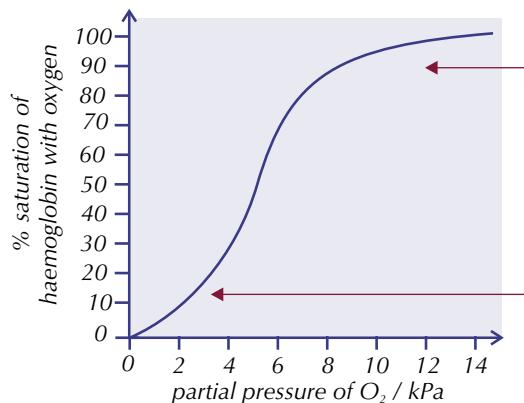


Figure 4: Dissociation curve for adult haemoglobin.

Weirdly, the saturation of haemoglobin can also affect the affinity — this is why the graph is 'S-shaped' and not a straight line.

When haemoglobin combines with the first O_2 molecule, its shape alters in a way that makes it easier for other O_2 molecules to join too. But as the haemoglobin starts to become saturated, it gets harder for more oxygen molecules to join. As a result, the curve has a steep bit in the middle where it's really easy for oxygen molecules to join, and shallow bits at each end where it's harder — see Figure 5. When the curve is steep, a small change in pO_2 causes a big change in the amount of oxygen carried by the haemoglobin.

Where pO_2 is high (e.g. in the lungs), haemoglobin has a high affinity for oxygen, so it has a high saturation of oxygen.

Where pO_2 is low (e.g. in respiring tissues), haemoglobin has a low affinity for oxygen, so it has a low saturation of oxygen.

Tip: These curves are sometimes called oxyhaemoglobin dissociation curves.

Tip: 100% saturation means every haemoglobin molecule is carrying the maximum of 4 molecules of oxygen.

Tip: 0% saturation means none of the haemoglobin molecules are carrying any oxygen.

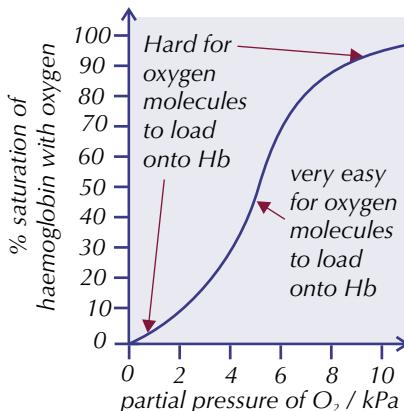


Figure 5: The S-shaped dissociation curve for haemoglobin.

Tip: kPa (kilopascal) is a unit used to measure pressure.

Carbon dioxide concentration

Tip: The word equation for respiration is:
glucose + oxygen → carbon dioxide + water + energy.

Tip: When dissociation curves are being compared, the further left the curve is, the higher the haemoglobin's affinity for oxygen is.

The **partial pressure of carbon dioxide (pCO_2)** is a measure of the concentration of CO_2 in a cell. To complicate matters, pCO_2 also affects oxygen unloading. Haemoglobin gives up its oxygen more readily at a higher pCO_2 . It's a cunning way of getting more O_2 to cells during activity.

When cells respire they produce carbon dioxide, which raises the pCO_2 . This increases the rate of oxygen unloading (i.e. the rate at which oxyhaemoglobin dissociates to form haemoglobin and oxygen) — so the dissociation curve 'shifts' right (but it stays the same shape). The saturation of blood with oxygen is lower for a given pO_2 , meaning that more oxygen is being released — see Figure 6. This is called the **Bohr effect**.

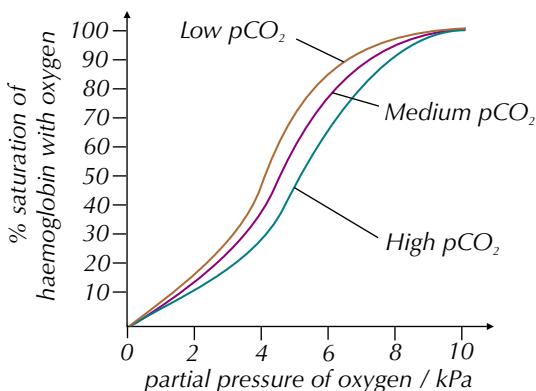


Figure 6: The Bohr effect.

Different types of haemoglobin

Different organisms have different types of haemoglobin with different oxygen transporting capacities — it depends on things like where they live, how active they are and their size. Having a particular type of haemoglobin is an adaptation that helps the organism to survive in a particular environment.

Tip: Environments with a low oxygen concentration include underground, at high altitudes or close to the seabed.

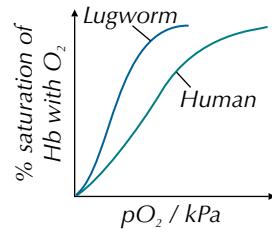
Tip: The curve shifts to the left for organisms that live in low oxygen environments. The curve shifts to the right for organisms that have a high respiration rate.

Low oxygen environments

Organisms that live in environments with a low concentration of oxygen have haemoglobin with a higher affinity for oxygen than human haemoglobin. This is because there isn't much oxygen available, so the haemoglobin has to be very good at loading any available oxygen. The dissociation curve of their haemoglobin is to the left of ours.

Example

A lugworm lives in burrows beneath sand where there's a low oxygen concentration. Its haemoglobin has to be able to pick up as much oxygen as possible — it has a high affinity for oxygen.



High activity levels

Organisms that are very active and have a high oxygen demand have haemoglobin with a lower affinity for oxygen than human haemoglobin. This is because they need their haemoglobin to easily unload oxygen, so that it's available for them to use. The dissociation curve of their haemoglobin is to the right of the human one.

Example

A hawk has a high respiratory rate (because it is very active) and lives where there's plenty of oxygen. Its haemoglobin has to be able to unload oxygen quickly in order to meet the high oxygen demand — it has a low affinity for oxygen.

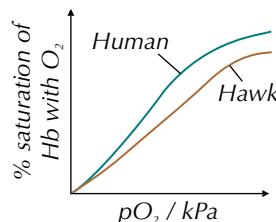


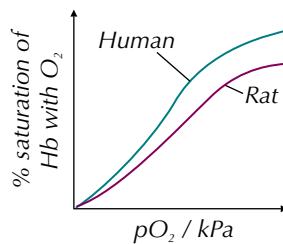
Figure 7: Hawks have a high oxygen demand.

Size

Small mammals tend to have a higher surface area to volume ratio than larger mammals. This means they lose heat quickly, so they have a high metabolic rate to help them keep warm — which means they have a high oxygen demand. Mammals that are smaller than humans have haemoglobin with a lower affinity for oxygen than human haemoglobin, because they need their haemoglobin to easily unload oxygen to meet their high oxygen demand. The dissociation curve of their haemoglobin is to the right of the human one.

Example

A rat has a higher surface area to volume ratio than a human. Its haemoglobin needs to unload oxygen easily to meet the greater oxygen demand — it has a lower affinity for oxygen.



Tip: See page 139 for more on surface area to volume ratios.

Tip: Metabolic rate is the rate at which energy is used. A higher metabolic rate leads to a higher respiration rate. This in turn leads to a higher oxygen demand.

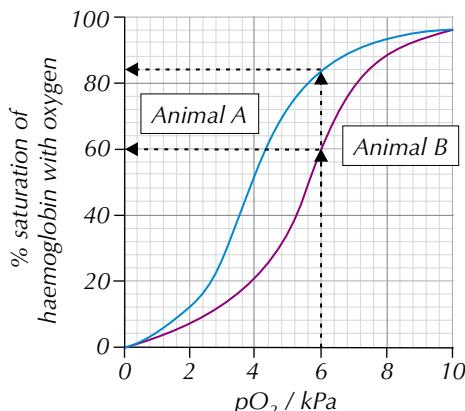
Reading values from a dissociation curve

In the exam, you might need to interpret a dissociation curve to answer a question. It's really important you can read data points off a graph correctly.

Example — Maths Skills

The graph on the right shows the oxygen dissociation curves for two animals. At a pO_2 of 6 kPa, what is the difference between the percentage saturation of haemoglobin with oxygen for the two animals?

1. Find 6 kPa on the x-axis.
2. Use a ruler to draw a line up to the curve for animal A. Make sure the line is parallel to the y-axis.
3. Then draw a line across to the y-axis. Make sure this line is parallel to the x-axis.
4. Read off the value at this point on the axis — 84%.
5. Repeat for the curve for animal B — 60%.
6. Find the difference between them: $84 - 60 = 24\%$



Exam Tip

Graphs crop up a lot in Biology exams. If you're shown one then asked to do a calculation or interpret the data, be aware that you'll probably need to find specific data points from the graph.

Exam Tip

You need to read graphs as accurately as you can — check the scales and the units on the axes very carefully to avoid throwing away marks.

Practice Questions — Application

Tip: To help you answer Q1 a), think of the difference in respiration rates between the two activities.

Exam Tip

In the exam you could be asked to interpret dissociation curves from animals you've never even heard of — don't let that throw you. Examiners want to make sure you really know your stuff by applying your knowledge to new situations.

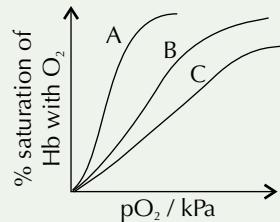
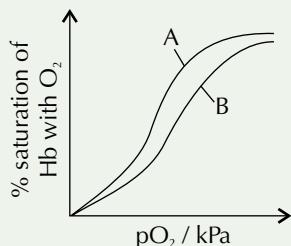
- Q1 The graph to the right shows two oxygen dissociation curves for the same man. One curve was produced based on blood tests when he was watching television and the other was produced based on blood tests immediately after a bike ride.

a) Which curve was produced after the bike ride? Explain your answer.

b) What name is given to the effect shown on the graph?

- Q2 The table below gives descriptions of three similarly sized animals. The graph shows each animal's dissociation curve. Match each animal to its dissociation curve and give reasons for your choices.

| Animal | Description |
|----------------------|-----------------------------------|
| Badger | Lives in an underground sett |
| Bush dog | Lives above ground, fairly active |
| Brown-throated sloth | Lives above ground, very inactive |



Practice Questions — Fact Recall

- Q1 What is the role of haemoglobin?
- Q2 Where is haemoglobin found in humans?
- Q3 How many polypeptide chains does a haemoglobin molecule have?
- Q4 Describe what is meant by haemoglobin 'loading' and 'unloading' oxygen.
- Q5 What is formed when oxygen is loaded onto haemoglobin?
- Q6 What is shown on an oxygen dissociation curve?
- Q7 Why does the binding of a single oxygen molecule increase haemoglobin's affinity for oxygen?
- Q8 Where in the body would you find cells with a high pO_2 ? Explain your answer.

3. The Circulatory System

The mammalian circulatory system is a mass transport system — it carries raw materials, as well as waste products, around the body of the mammal.

Function of the circulatory system

Multicellular organisms, like mammals, have a low surface area to volume ratio (see p. 141), so they need a specialised mass transport system to carry raw materials from specialised exchange organs to their body cells — this is the circulatory system.

Structure of the circulatory system

The circulatory system is made up of the heart and blood vessels. The heart pumps blood through blood vessels (**arteries**, **arterioles**, **veins** and **capillaries**) to reach different parts of the body. You need to know the names of the blood vessels entering and leaving the heart, lungs and kidneys. These are shown in Figures 1 and 2.

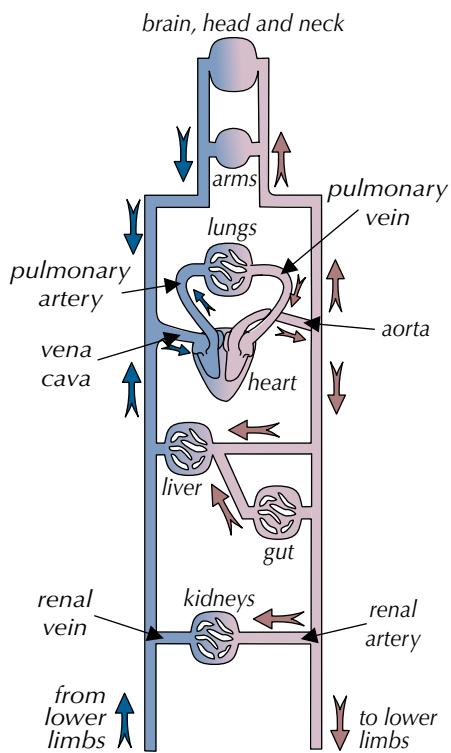


Figure 1: The circulatory system.

Blood transports respiratory gases, products of digestion, metabolic wastes and hormones round the body. There are two circuits. One circuit takes blood from the heart to the lungs, then back to the heart. The other loop takes blood around the rest of the body, so the blood has to go through the heart twice to complete one full circuit of the body. The heart has its own blood supply — the left and right **coronary arteries** — see Figure 3.

| Vessel | Carries blood from... | Carries blood to... |
|------------------|-----------------------|---------------------|
| Pulmonary artery | heart | lungs |
| Pulmonary vein | lungs | heart |
| Aorta | heart | body |
| Vena cava | body | heart |
| Renal artery | body | kidneys |
| Renal vein | kidneys | vena cava |

Figure 2: Some of the blood vessels in a mammalian circulatory system.

Learning Objectives:

- Know the general pattern of blood circulation in a mammal.
- Know the names of the coronary arteries and the blood vessels entering and leaving the heart, lungs and kidneys.
- Know the structure of arteries, arterioles and veins in relation to their function.
- Know the structure of capillaries and the importance of capillary beds as exchange surfaces.
- Understand how tissue fluid is formed and how it is returned to the circulatory system.

Specification Reference 3.3.4.1

Tip: Blood always flows from a higher pressure to a lower pressure in the circulatory system. The vena cava is the final blood vessel that takes the blood back to the heart from the body, so it has the lowest pressure.

Tip: The gut is another name for the digestive tract or a part of it, e.g. the intestines.

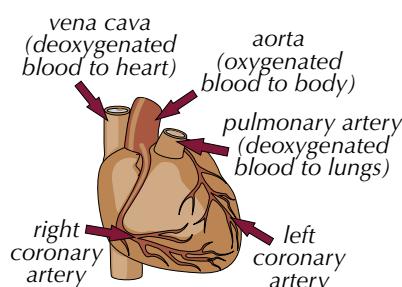


Figure 3: Blood vessels of the heart.

Tip: When you're looking at a diagram of a heart, imagine it's in the body of someone standing opposite you, so the left and right sides are opposite to your left and right.

Arteries, arterioles and veins

Arteries, arterioles and veins have different characteristics, and you need to know why...

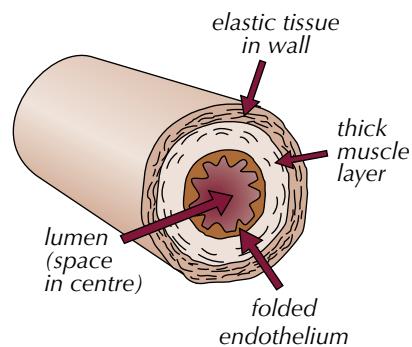
Arteries

Tip: Arteries are the 'way out' (way out) of the heart, and veins are the 'vey in' (way in).

Exam Tip

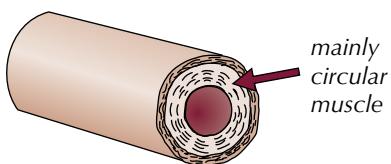
An artery stretches to cope with high pressure and then recoils under low pressure — you won't get marks for writing that it contracts and relaxes, or expands.

Arteries carry blood from the heart to the rest of the body. Their walls are thick and muscular and have elastic tissue to stretch and recoil as the heart beats, which helps maintain the high pressure. The inner lining (called the endothelium) is folded, allowing the artery to stretch — this also helps it to maintain high pressure. All arteries carry oxygenated blood except for the pulmonary arteries, which take deoxygenated blood to the lungs.



Arterioles

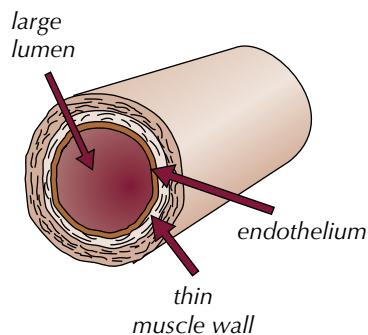
Arteries divide into smaller vessels called arterioles. These form a network throughout the body. Blood is directed to different areas of demand in the body by muscles inside the arterioles, which contract to restrict the blood flow or relax to allow full blood flow.



Veins

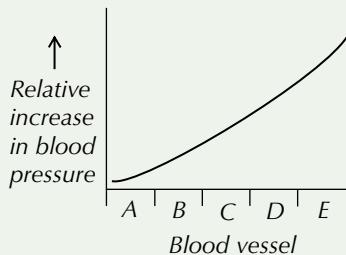
Tip: The pressure decreases along a blood vessel due to friction.

Veins take blood back to the heart under low pressure. They have a wider lumen than equivalent arteries, with very little elastic or muscle tissue. Veins contain **valves** to stop the blood flowing backwards (see page 180). Blood flow through the veins is helped by contraction of the body muscles surrounding them. All veins carry deoxygenated blood (because oxygen has been used up by body cells), except for the pulmonary veins, which carry oxygenated blood to the heart from the lungs.



Practice Question — Application

- Q1 The graph on the right shows the relative increase in blood pressure in different blood vessels of a mammal's circulatory system.

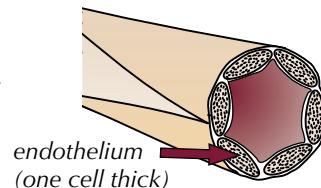


Measurements were taken in the renal artery, the renal vein, an arteriole, the aorta and the vena cava.

Suggest which letter represents each blood vessel. Explain your choices.

Capillaries

Arterioles branch into capillaries, which are the smallest of the blood vessels. Substances (e.g. glucose and oxygen) are exchanged between cells and capillaries, so they're adapted for efficient diffusion. Capillaries are always found very near cells in exchange tissues (e.g. alveoli in the lungs), so there's a short diffusion pathway. Their walls are only one cell thick, which also shortens the diffusion pathway. There are a large number of capillaries, to increase surface area for exchange. Networks of capillaries in tissue are called **capillary beds**.



Tip: Capillaries connect arterioles and venules together at capillary beds. Venules are small blood vessels that connect to veins.

Tissue fluid

Tissue fluid is the fluid that surrounds cells in tissues. It's made from small molecules that leave the blood plasma, e.g. oxygen, water and nutrients. (Unlike blood, tissue fluid doesn't contain red blood cells or big proteins, because they're too large to be pushed out through the capillary walls.) Cells take in oxygen and nutrients from the tissue fluid, and release metabolic waste into it. In a capillary bed, substances move out of the capillaries, into the tissue fluid, by **pressure filtration**.

At the start of the capillary bed, nearest the arteries, the hydrostatic (liquid) pressure inside the capillaries is greater than the hydrostatic pressure in the tissue fluid. This difference in hydrostatic pressure means an overall outward pressure forces fluid out of the capillaries and into the spaces around the cells, forming tissue fluid. As fluid leaves, the hydrostatic pressure reduces in the capillaries — so the hydrostatic pressure is much lower at the venule end of the capillary bed (the end that's nearest to the veins).

Due to the fluid loss, and an increasing concentration of plasma proteins (which don't leave the capillaries), the water potential at the venule end of the capillary bed is lower than the water potential in the tissue fluid. This means that some water re-enters the capillaries from the tissue fluid at the venule end by osmosis (see p. 106 for more on osmosis). Any excess tissue fluid is drained into the **lymphatic system** (a network of tubes that acts a bit like a drain), which transports this excess fluid from the tissues and passes it back into the circulatory system.

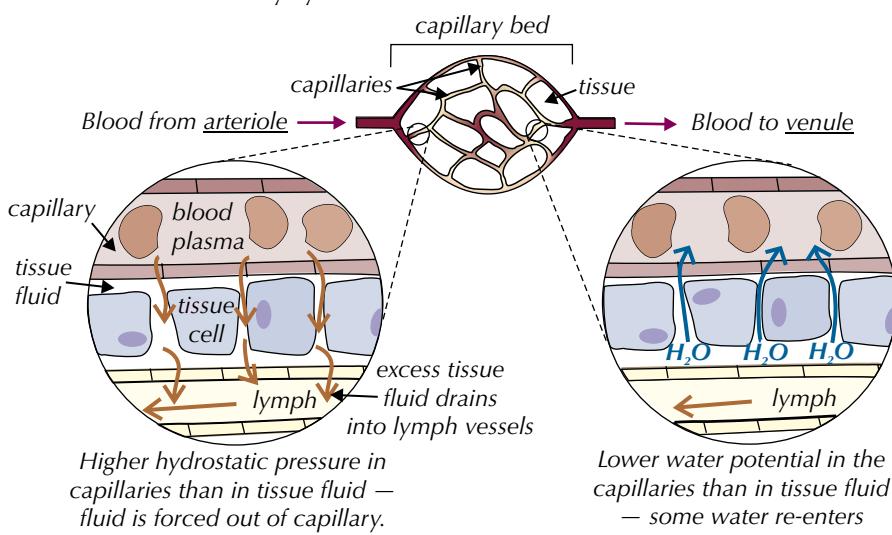


Figure 4: The movement of fluid between capillaries, tissue fluid and lymph.

Tip: Blood plasma is just the liquid that carries everything in the blood.

Exam Tip

Don't write in the exam that tissue fluid doesn't contain any proteins — it still contains some, just not big ones.

Tip: Pressure filtration is just what it sounds like — filtration happening under pressure. Here it describes the process by which small molecules are filtered out of the capillaries under hydrostatic pressure, forming tissue fluid.

Tip: Pressure is highest at the start of a capillary bed nearest the arterioles — this is caused by the left ventricle contracting and sending the blood out of the heart, through the arteries and arterioles, at high pressure.

Tip: High blood pressure means a high hydrostatic pressure in the capillaries, which can lead to an accumulation of tissue fluid in the tissues.

Practice Questions — Application

- Q1 A scientist recorded the hydrostatic pressure of blood and tissue fluid at two points along a capillary bed. The results are shown in the table below.

| | Pressure at Point A (kPa) | Pressure at Point B (kPa) |
|--------------|---------------------------|---------------------------|
| Blood | 2 | 3.5 |
| Tissue fluid | 4 | 2 |

- a) The direction of fluid movement between blood and tissue fluid changes along the capillary.
- Where does fluid move from and to at point B?
 - What does the fluid contain at point B?
- b) Suggest where on the capillary bed you would find:
- point A
 - point B

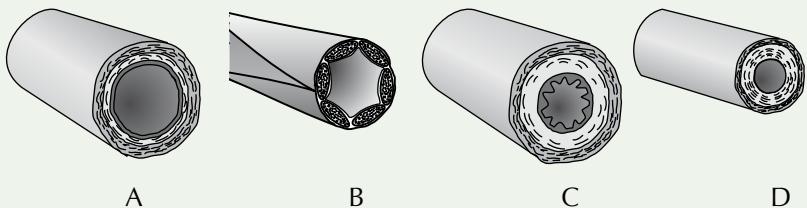
- Q2 Albumin is a protein found in the blood. Hypoalbuminemia is a condition where the level of albumin in the blood is very low. It causes an increase in tissue fluid, which can lead to swelling. Explain how hypoalbuminemia causes an increase in tissue fluid.

Tip: For Q2, think about how the concentration of protein in the blood affects the water potential of the capillary.

Tip: To distinguish between different types of blood vessels, you need to think about how thick the walls need to be for each types and how much muscle there will be in the walls, etc.

Practice Questions — Fact Recall

- Q1 Why do mammals need a circulatory system?
- Q2 Name the two blood vessels that carry blood into the heart.
- Q3 Which blood vessel carries deoxygenated blood to the lungs?
- Q4 Which blood vessel carries blood to the kidneys?
- Q5 Which vessels supply the heart tissue with blood?
- Q6 Name the blood vessels A - D shown below.
(The diagrams are not drawn to scale).



- Q7 Describe the structure of an artery.
- Q8 What is an arteriole?
- Q9 a) Name the blood vessels that have valves in them.
b) What is the function of these valves?
- Q10 Give two ways in which capillaries are adapted for efficient diffusion.
- Q11 What is tissue fluid?
- Q12 a) Explain the movement of fluid at the arteriole end of a capillary bed.
b) Explain the movement of water at the venule end of a capillary bed.

4. The Heart

Your heart is responsible for pumping blood all around your body, through your blood vessels. So it's quite important really...

The structure of the heart

Figure 1 below shows the internal structure of the heart. The right side pumps deoxygenated blood to the lungs and the left side pumps oxygenated blood to the whole body. Note — the left and right sides are reversed on the diagram, cos it's the left and right of the person that the heart belongs to.

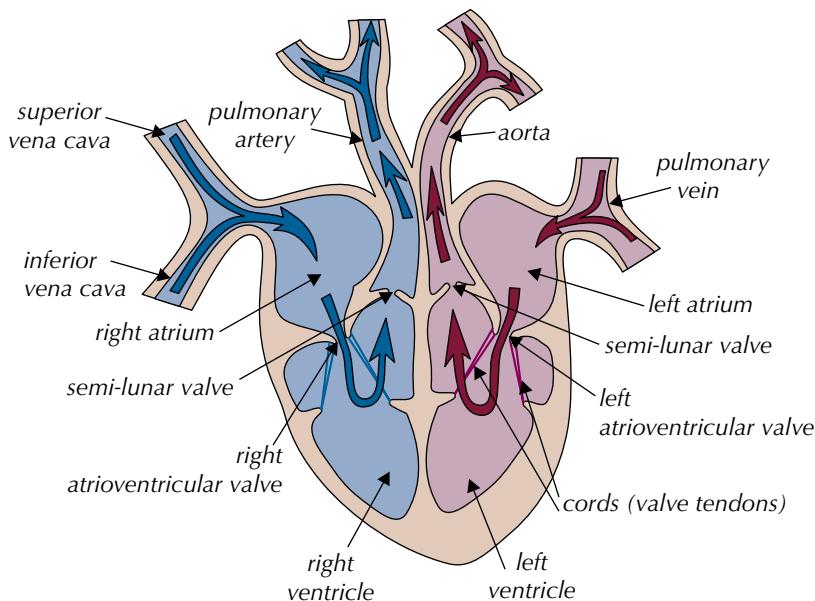


Figure 1: The internal structure of the heart.

Each bit of the heart is adapted to do its job effectively.

- The **left ventricle** of the heart has thicker, more muscular walls than the right ventricle — this allows it to contract more powerfully and pump blood all the way around the body. The right side is less muscular so its contractions are only powerful enough to pump blood to the nearby lungs.
- The **ventricles** have thicker walls than the atria therefore they can push blood out of the heart, whereas the atria just need to push blood a short distance into the ventricles.
- The **atrioventricular (AV) valves** link the atria to the ventricles and stop blood flowing back into the atria when the ventricles contract.
- The **semi-lunar (SL) valves** link the ventricles to the pulmonary artery and aorta, and stop blood flowing back into the heart after the ventricles contract.
- The **cords** attach the atrioventricular valves to the ventricles to stop them being forced up into the atria when the ventricles contract.

Learning Objectives:

- Know the gross structure of the human heart.
- Be able to dissect an organ within an animal's mass transport system (Required Practical 5).
- Understand the pressure and volume changes and associated valve movements during the cardiac cycle that maintain a unidirectional flow of blood.
- Be able to analyse and interpret data relating to pressure and volume changes during the cardiac cycle.

Specification Reference 3.3.4.1

Tip: The diagram is a good reminder that veins carry blood into the heart (vena cava and pulmonary vein) and arteries carry blood away from it (pulmonary artery and aorta).

Tip: Right side of the heart = deoxygenated blood (blue). Left side of the heart = oxygenated blood (pink).

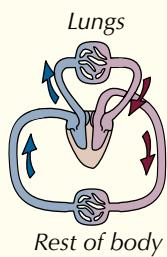




Figure 2: A heart valve.

Tip: Remember to carry out a risk assessment before you begin your dissection — be especially careful with sharp dissection tools.

Tip: You're likely to be given a pig or cow's heart to dissect.

Tip: See page 160 for more information on dissections and the tools that you might need to use.



Figure 4: A heart before dissection. The fat on the outside may make it hard to see the openings of the blood vessels — you might have to find them with your fingers.

Heart valves

The valves only open one way — whether they're open or closed depends on the relative pressure of the heart chambers. If there's higher pressure behind a valve, it's forced open, but if pressure is higher in front of the valve it's forced shut — see Figure 3. This means that the flow of blood is unidirectional — it only flows in one direction.

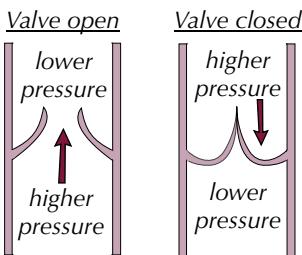


Figure 3:
Diagram showing
how heart valves
open and close.

REQUIRED PRACTICAL 5

Heart dissection

As one of your required practicals you may need to dissect an organ within an animal's mass transport system. If you're lucky enough to get a heart to dissect, this is how you'd do it:

1. Make sure you are wearing a lab coat and lab gloves because heart dissections can be messy.
2. Place the heart you are given on your dissecting tray.
3. Look at the outside of the heart and try to identify the four main vessels attached to it. Feel inside the vessels to help you — remember arteries are thick and rubbery, whereas veins are much thinner.
4. Identify the right and left atria, the right and left ventricles and the coronary arteries. You might be asked to draw a sketch of the outside of the heart and label it.
5. Using a clean scalpel, carefully cut along the lines shown on Figure 5 to look inside each ventricle. You could measure and record the thickness of the ventricle walls and note any differences between them.
6. Next, cut open the atria and look inside them too. Note whether the atria walls are thicker or thinner than the ventricle walls.
7. Then find the atrioventricular valves, followed by the semi-lunar valves. Look at the structure of the valves and see if you can see how they only open one way. Again, you could draw a sketch to show the valves and the inside of the ventricles and atria.
8. Make sure you wash your hands and disinfect all work surfaces once you've completed your dissection.

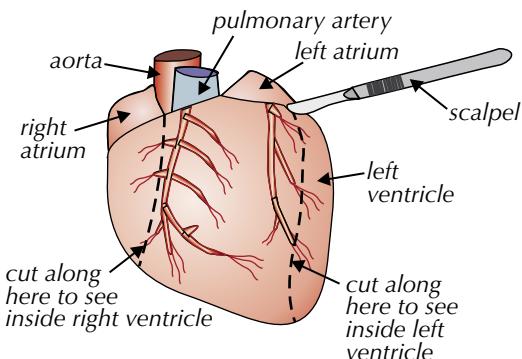


Figure 5:
Diagram showing
where to cut
heart to examine
the ventricles.

The cardiac cycle

The cardiac cycle is an ongoing sequence of contraction and relaxation of the atria and ventricles that keeps blood continuously circulating round the body. The volume of the atria and ventricles changes as they contract and relax. Pressure changes also occur, due to the changes in chamber volume (e.g. decreasing the volume of a chamber by contraction will increase the pressure of a chamber). The cardiac cycle can be simplified into three stages:

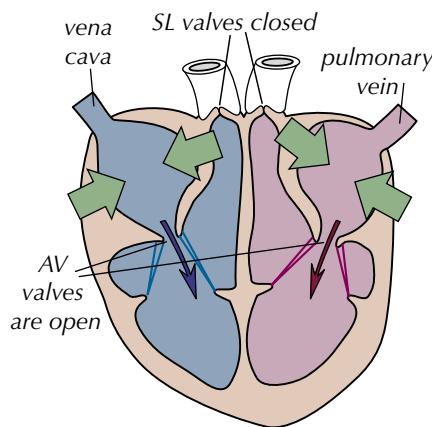
Tip: Cardiac contraction is also called systole, and relaxation is called diastole.

1. Ventricles relax, atria contract

The ventricles are relaxed.

The atria contract, decreasing the volume of the chambers and increasing the pressure inside the chambers.

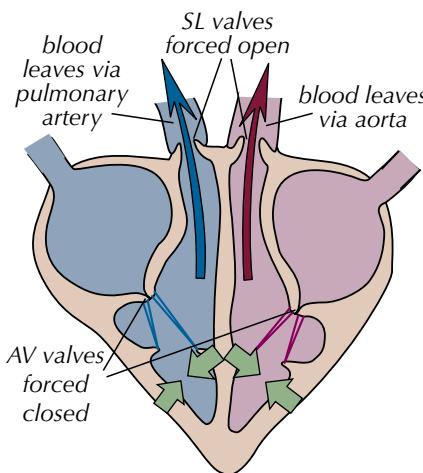
This pushes the blood into the ventricles. There's a slight increase in ventricular pressure and chamber volume as the ventricles receive the ejected blood from the contracting atria.



Tip: Contraction of the atria or ventricles is a bit like squeezing a balloon — the size of the balloon decreases and the pressure inside it increases.

2. Ventricles contract, atria relax

The atria relax. The ventricles contract (decreasing their volume), increasing their pressure. The pressure becomes higher in the ventricles than the atria, which forces the atrioventricular (AV) valves shut to prevent back-flow. The pressure in the ventricles is also higher than in the aorta and pulmonary artery, which forces open the semi-lunar (SL) valves and blood is forced out into these arteries.

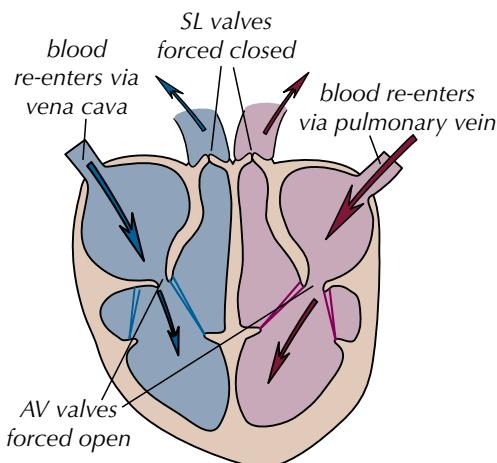


Tip: Remember that if there's a higher pressure in front of a valve it's forced shut and if there's a higher pressure behind a valve it's forced open (see previous page).

3. Ventricles relax, atria relax

The ventricles and the atria both relax. The higher pressure in the pulmonary artery and aorta closes the SL valves to prevent back-flow into the ventricles.

Blood returns to the heart and the atria fill again due to the higher pressure in the vena cava and pulmonary vein. In turn this starts to increase the pressure of the atria. As the ventricles continue to relax, their pressure falls below the pressure of the atria and so the AV valves open. This allows blood to flow passively (without being pushed by atrial contraction) into the ventricles from the atria. The atria contract, and the whole process begins again.



Exam Tip

When writing about the cardiac cycle in the exam, make sure you always name the valves. You should also make sure you name them in full at least once before abbreviating them.

Tip: Remember that it's the change in volume in a chamber that causes the change in pressure — see previous page.

Interpreting data on the cardiac cycle

You may well be asked to analyse or interpret data about the changes in pressure and volume during the cardiac cycle. Here are two examples of the kind of things you might get:

Example 1

If you get a graph you could be asked questions like this:

When does blood start flowing into the aorta?

At point A, the ventricles are contracting, which increases the pressure inside them. Once the pressure inside the ventricles is higher than that in the atria, the atrioventricular valves shut, forcing blood into the aorta.

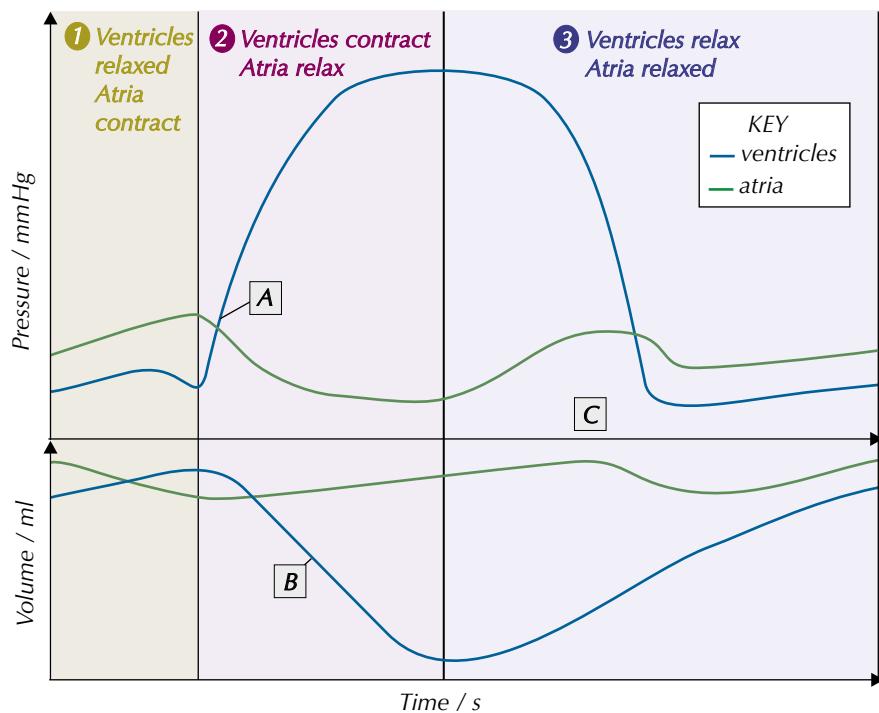
Why is ventricular volume decreasing at point B?

The ventricles are contracting, reducing the volume of the chamber.

Are the semi-lunar valves open or closed at point C? Closed.

The ventricles are relaxed and refilling, so the pressure is higher in the pulmonary artery and aorta, forcing the SL valves closed.

Tip: mmHg is a unit of measurement for pressure. It means millimetres of mercury.



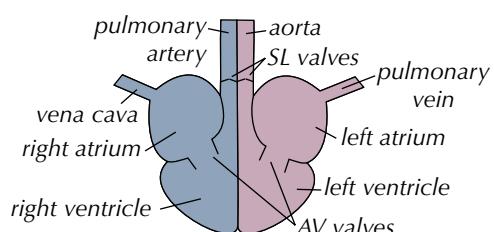
Exam Tip

In the exam, the heart might not always be drawn like the one we've shown on the right. Don't let this throw you — just look to see where the valves are and whether they're opened or closed, then answer the questions.

Example 2

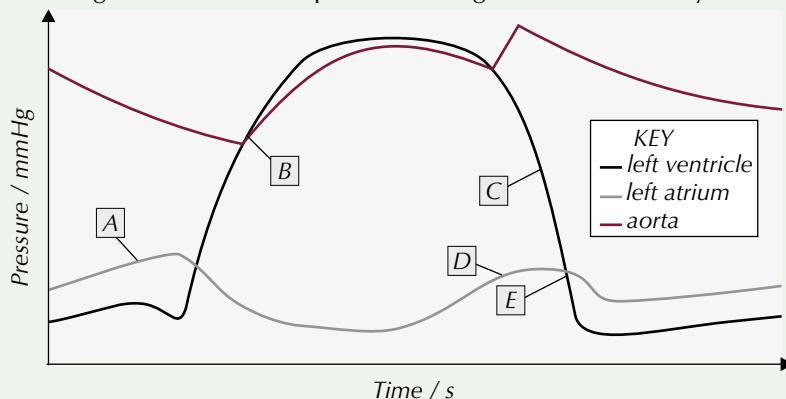
You may have to describe the changes in pressure and volume shown by a diagram, like the one below. In this diagram the AV valves are open.

So you know that the pressure in the atria is higher than in the ventricles. So the atria must be contracting because that's what causes the increase in pressure.



Practice Questions — Application

The diagram below shows pressure changes in the cardiac cycle.



- Q1 Why is the atrial pressure increasing at point A?
- Q2 Is the semi-lunar valve open or closed at point B? Explain your answer.
- Q3 Why is the ventricular pressure decreasing at point C?
- Q4 Why is the atrial pressure increasing at point D?
- Q5 Is the atrioventricular valve open or closed at point E? Explain your answer.

Tip: The left ventricle has a thicker wall than the right ventricle and so it contracts more forcefully. This means the pressure is higher in the left ventricle (and in the aorta).

Exam Tip

In the exam, if you're given a graph like the one on the left, make sure you read the key carefully so that you don't get the lines mixed up and answer the question incorrectly.

Calculating cardiac output

Examiners love throwing calculations into the exam, and this Topic is no exception. For example, you could be asked to calculate cardiac output (CO).

Cardiac output is the volume of blood pumped by the heart per minute (measured in $\text{cm}^3 \text{ min}^{-1}$). It's calculated using this formula:

$$\text{cardiac output} = \text{stroke volume} \times \text{heart rate}$$

- **Heart rate** — the number of beats per minute (bpm).
- **Stroke volume** — the volume of blood pumped during each heartbeat, measured in cm^3 .

Examples — Maths Skills

- Calculate your **cardiac output** if you have a stroke volume of 70 cm^3 and a heart rate of 75 bpm.

$$\begin{aligned}\text{cardiac output} &= \text{stroke volume} \times \text{heart rate} \\ &= 70 \times 75 = 5250 \text{ cm}^3 \text{ min}^{-1}\end{aligned}$$

- Calculate your **stroke volume** if you have a heart rate of 80 bpm and a cardiac output of $5440 \text{ cm}^3 \text{ min}^{-1}$.

$$\text{stroke volume} = \frac{\text{cardiac output}}{\text{heart rate}} = \frac{5440}{80} = 68 \text{ cm}^3$$

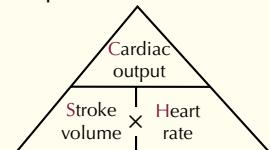
- Calculate your **heart rate** if you have a stroke volume of 68 cm^3 and a cardiac output of $4896 \text{ cm}^3 \text{ min}^{-1}$.

$$\text{heart rate} = \frac{\text{cardiac output}}{\text{stroke volume}} = \frac{4896}{68} = 72 \text{ bpm}$$

Exam Tip

You don't need to learn this formula off by heart.

Tip: If you're struggling to remember how to change the subject of a formula, then a formula triangle like this might help:



To use a formula triangle, put your finger over the bit of the triangle that corresponds to what you want to find, then read off the correct formula.

Practice Questions — Application

Exam Tip

Remember to always include the correct units in your answers to calculations.

The formula for calculating cardiac output is given below. Use it to answer the questions that follow.

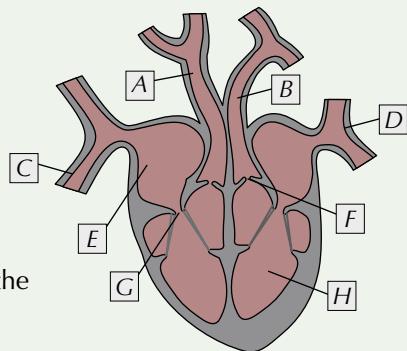
$$\text{cardiac output} = \text{stroke volume} \times \text{heart rate}$$

- Q1 If you have a stroke volume of 61 cm^3 and a heart rate of 79 bpm, what is your cardiac output?
- Q2 If you have a stroke volume of 72.5 cm^3 and a cardiac output of $5075 \text{ cm}^3 \text{ min}^{-1}$, what is your heart rate?
- Q3 If you have a heart rate of 75 bpm and a cardiac output of $5175 \text{ cm}^3 \text{ min}^{-1}$, what is your stroke volume?

Practice Questions — Fact Recall

Tip: To answer Q3 on the right, think about where the left and right ventricles are pumping blood to.

- Q1 Which side of the heart pumps deoxygenated blood?
- Q2 The diagram on the right shows the heart. Name the structures labelled A to H.
- Q3 Why does the left ventricle of the heart have thicker, more muscular walls than the right ventricle?
- Q4
 - a) Name the valves that link the ventricles to the aorta and pulmonary artery.
 - b) What is the function of these valves?
- Q5 What is the cardiac cycle?
- Q6 When the atria contract, describe the pressure and volume changes that take place in the atria.



5. Cardiovascular Disease

Your circulatory system keeps you going by constantly supplying all parts of your body with oxygen and glucose for respiration. However, sometimes things can go wrong...

What is cardiovascular disease?

Cardiovascular disease is a general term used to describe diseases associated with the heart and blood vessels. Cardiovascular diseases include aneurysms, thrombosis and myocardial infarction — see next page. Most cardiovascular disease starts with atheroma formation (see below).

Coronary heart disease (CHD) is a type of cardiovascular disease. It occurs when the coronary arteries have lots of atheromas in them, which restricts blood flow to the heart muscle. It can lead to myocardial infarction.

Atheroma formation

The wall of an artery is made up of several layers (see p. 176). The endothelium (inner lining) is usually smooth and unbroken. If damage occurs to the endothelium (e.g. by high blood pressure), white blood cells (mostly macrophages) and lipids (fat) from the blood, clump together under the lining to form fatty streaks.

Over time, more white blood cells, lipids and connective tissue build up and harden to form a fibrous plaque called an atheroma — see Figure 2. This plaque partially blocks the lumen of the artery and restricts blood flow, which causes blood pressure to increase.

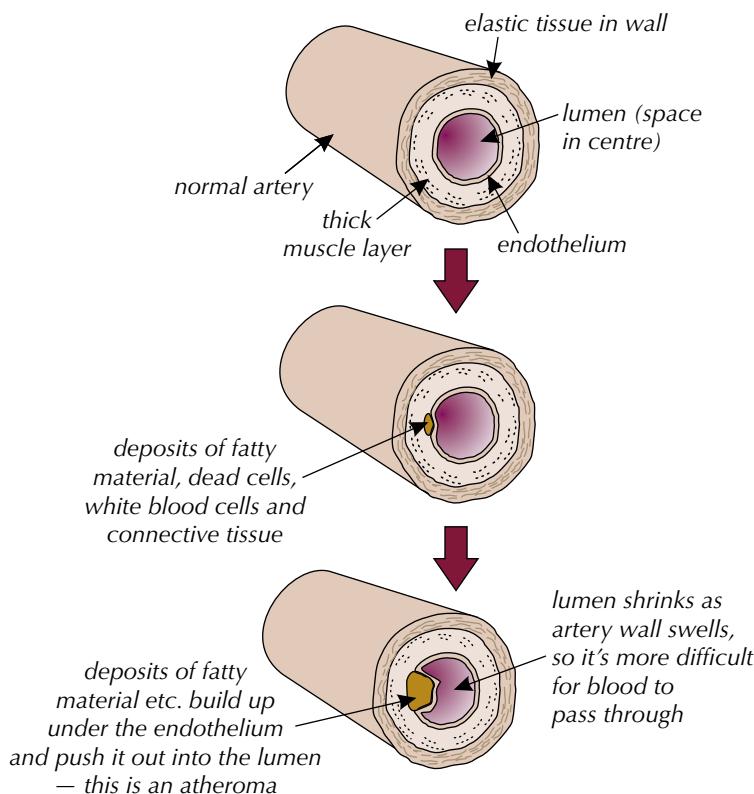


Figure 2: The process of atheroma formation.

Learning Objectives:

- Be able to analyse and interpret data associated with specific risk factors and the incidence of cardiovascular disease.
- Be able to recognise correlations and causal relationships.
- Be able to evaluate conflicting evidence associated with risk factors affecting cardiovascular disease.

Specification Reference 3.3.4.1

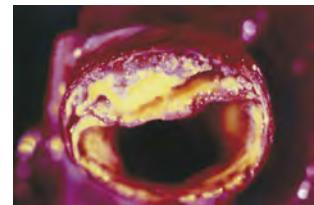


Figure 1: An atheroma inside an artery.

Tip: Some people are more at risk of developing atheromas than others (see pages 187-188 for more).



Figure 3: An x-ray of an aneurysm (red balloon) in the aorta.

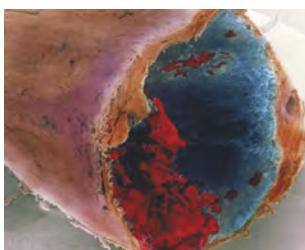


Figure 4: A blood clot in an artery.

Tip: You don't need to learn the details of each type of cardiovascular disease, but it will help with interpreting data about them if you understand what is involved in each case.

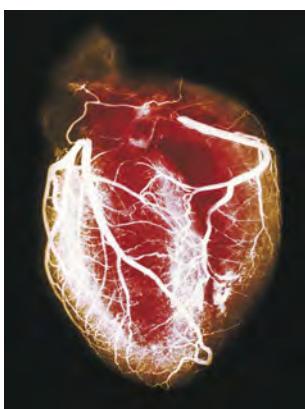
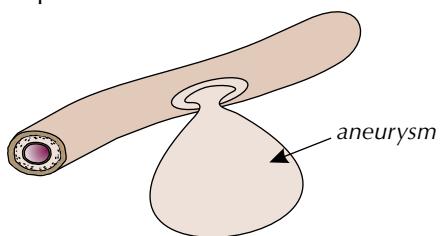


Figure 6: The coronary arteries.

Aneurysm

An aneurysm is a balloon-like swelling of the artery. It starts with the formation of atheromas. Atheroma plaques damage and weaken arteries. They also narrow arteries, increasing blood pressure. When blood travels through a weakened artery at high pressure, it may push the inner layers of the artery through the outer elastic layer to form an aneurysm. This aneurysm may burst, causing a haemorrhage (bleeding).



Thrombosis

Thrombosis is the formation of a blood clot. It also starts with the formation of atheromas. An atheroma plaque can rupture (burst through) the endothelium (inner lining) of an artery. This damages the artery wall and leaves a rough surface. Platelets and fibrin (a protein) accumulate at the site of damage and form a **blood clot** (a thrombus). This blood clot can cause a complete blockage of the artery, or it can become dislodged and block a blood vessel elsewhere in the body. Debris from the rupture can cause another blood clot to form further down the artery.

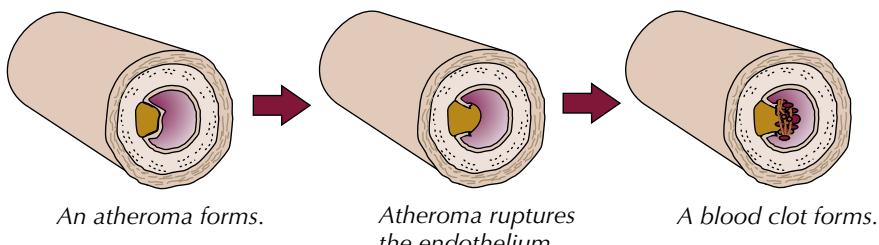


Figure 5: Formation of a blood clot.

Myocardial infarction (heart attack)

The heart muscle is supplied with blood by the **coronary arteries** — see Figure 6. This blood contains the oxygen needed by heart muscle cells to carry out respiration. If a coronary artery becomes completely blocked (e.g. by a blood clot) an area of the heart muscle will be totally cut off from its blood supply, receiving no oxygen. This causes a myocardial infarction — more commonly known as a heart attack — see Figure 7.

A heart attack can cause damage and death of the heart muscle. Symptoms include pain in the chest and upper body, shortness of breath and sweating. If large areas of the heart muscle are affected complete heart failure can occur, which is often fatal.

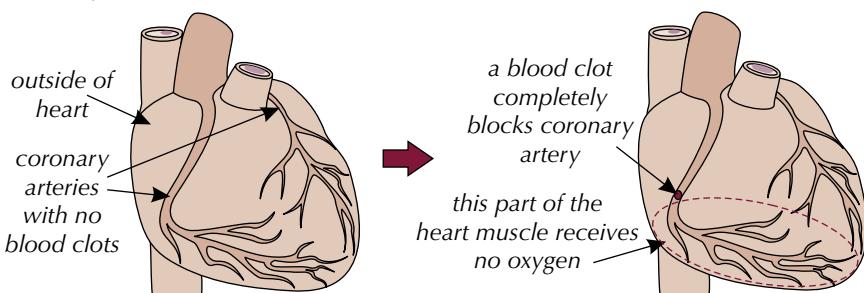


Figure 7: How a heart attack is caused.

Risk factors for cardiovascular disease

There are quite a few things that increase your risk of getting atherosomas in your arteries, like smoking or too much salt in your diet. Here are some of the most common risk factors and how they can lead to the development of a myocardial infarction:

Tip: A risk factor is something that increases your chance of developing a disease.

High blood pressure

High blood pressure increases the risk of damage to the artery walls. Damaged walls have an increased risk of atheroma formation, causing a further increase in blood pressure. Atherosomas can also cause blood clots to form (see previous page). A blood clot could block flow of blood to the heart muscle, possibly resulting in myocardial infarction (see previous page for details). So anything that increases blood pressure also increases the risk of cardiovascular disease, e.g. being overweight, not exercising and excessive alcohol consumption.



Figure 1: The link between high blood pressure, atheroma formation and myocardial infarction.

Exam Tip

It's not a good idea to write about high blood pressure 'putting a strain on the heart' — you need to be more technically accurate and write about it increasing the risk of damage to artery walls.

High blood cholesterol and poor diet

If the blood cholesterol level is high (above 240 mg per 100 cm³) then the risk of cardiovascular disease is increased. This is because cholesterol is one of the main constituents of the fatty deposits that form atherosomas. Atherosomas can lead to increased blood pressure and blood clots, which could cause a myocardial infarction.

Exam Tip

Don't refer to atherosomas 'furring up arteries' — they are fibrous plaques (containing fatty material) that narrow the lumen of arteries.

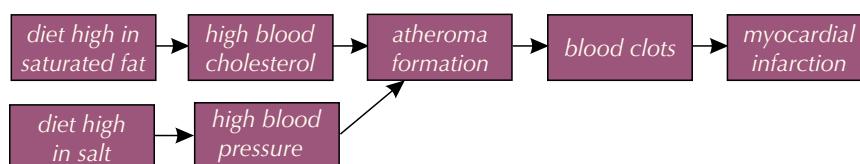


Figure 2: The link between a diet high in saturated fat or salt, atheroma formation and myocardial infarction.

Cigarette smoking

Both carbon monoxide and nicotine, found in cigarette smoke, increase the risk of cardiovascular disease and myocardial infarction.

Tip: Cardiovascular disease doesn't just affect the heart. For example, a blood clot in an artery in the brain can cause a stroke.

Carbon monoxide combines with haemoglobin and reduces the amount of oxygen transported in the blood, and so reduces the amount of oxygen available to tissues. If the heart muscle doesn't receive enough oxygen it can lead to a heart attack.

Smoking also decreases the amount of antioxidants in the blood — these are important for protecting cells from damage. Fewer antioxidants means cell damage in the coronary artery walls is more likely, and this can lead to atheroma formation.

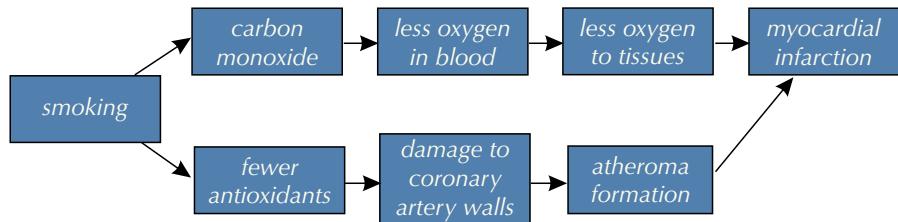


Figure 3: The link between smoking, atherosclerosis formation and myocardial infarction.

Tip: Other common risk factors for cardiovascular disease include obesity, a lack of physical activity, ethnic background (people of South Asian or African Caribbean background may have a greater risk of certain forms of CVD), age (risk increases with age) and sex (men are more at risk than women).

Exam Tip

Make sure you read any information you're given about a study really carefully — it will help you to interpret data from the study and decide how much confidence you can have in any conclusions made (see next page).

Reducing the risk

Most of these factors are within our control — a person can choose to smoke, eat fatty foods, etc. However, some risk factors can't be controlled, such as having a genetic predisposition to coronary heart disease or having high blood pressure as a result of another condition, e.g. some forms of diabetes. Even so, the risk of developing cardiovascular disease can be reduced by removing as many risk factors as you possibly can.

Interpreting data on risk factors and cardiovascular disease

Take a look at the following example of the sort of study you might see in your exam.

Example — LDL cholesterol level

Figure 4 shows the results of a study involving 27 939 American women. The LDL cholesterol level was measured for each woman. These women were then followed for an average of 8 years and the occurrence of cardiovascular events (e.g. heart attack, surgery on coronary arteries) or death from cardiovascular diseases was recorded. The relative risk of a cardiovascular event, adjusted for other factors that can affect cardiovascular disease, was then calculated.

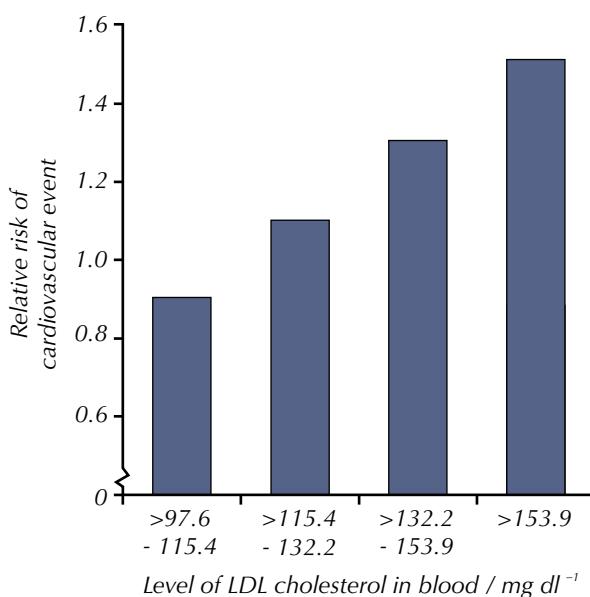


Figure 4: Graph showing the relationship between the level of LDL cholesterol in the blood and the relative risk of cardiovascular event.

Here are some of the things you might be asked to do:

1. **Describe the data** — The relative risk of a cardiovascular event increases as the level of LDL cholesterol in the blood increases, from 0.9 at $> 97.6 - 115.4 \text{ mg dl}^{-1}$ to 1.5 at $> 153.9 \text{ mg dl}^{-1}$.
2. **Draw conclusions** — The graph shows a positive correlation between the relative risk of a cardiovascular event and the level of LDL cholesterol in the blood.
3. **Check any conclusions are valid** — Make sure any conclusions match the data, e.g.
 - This data only looked at women — no males were involved, so you can't say that this trend is true for everyone.
 - You can't say that a high LDL cholesterol level is correlated with an increased risk of heart attacks, because the data shows all first cardiovascular events, including surgery on coronary arteries.
 - Also, you can't conclude that a high LDL cholesterol level caused the increased relative risk of a cardiovascular event — there may be other reasons for the trend.
4. **Other things to think about** — A large sample size was used (27 939). Data based on large samples is better than data based on small samples. This is because a large sample is more representative of the whole population (i.e. it shares more of the various characteristics of the population).

Tip: There's more on correlation and cause on page 15.

Tip: The way in which information is collected can also be important. Some studies rely on the results of questionnaires (e.g. asking people how many cigarettes they smoke). Questionnaires can be unreliable as people can tell fibs or give inaccurate information.

Conflicting evidence

You might also have to evaluate conflicting evidence associated with risk factors affecting cardiovascular disease. E.g. one study might conclude that a factor isn't a health risk, whereas another study might conclude that the same factor is a health risk.

If two studies have produced conflicting results, think about why that might be. Was it to do with study design? Was one study based on a small sample size? Did both studies take into account other risk factors (variables) that could have affected the results? Knowing whether both studies used similar groups can be helpful, e.g. same age, gender, etc.

Sometimes, the only way to resolve the problem of conflicting evidence is to carry out more studies and collect more results. Results need to be reproduced by other scientists before they're accepted.

Practice Question — Application

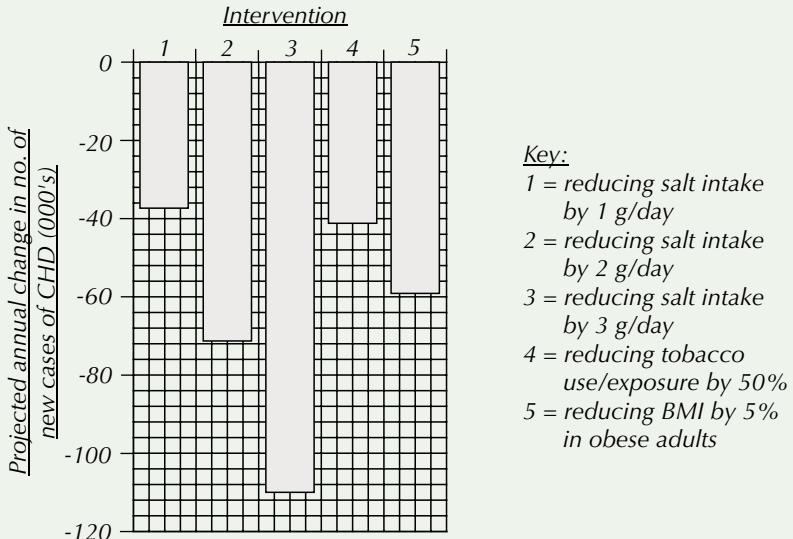
- Q1 In a US study, a computer model was used to predict how interventions to reduce some CHD risk factors could affect the number of new cases of CHD per year. The results are shown in the graph on the next page. The results for reducing salt intake are based on the highest estimates from the study.

Tip: Remember, CHD stands for coronary heart disease.

Tip: You need to be really careful when reading complex graphs — make sure you pay attention to the key and axes, so that you know exactly what the graph is showing you.

Tip: An intervention to reduce a risk factor could be a change in diet or lifestyle.

Tip: BMI (body mass index) is the relationship between weight and height — it's used as a measure of obesity.



- Describe the effect that reducing salt intake by 1 g per day could have on the number of new cases of CHD per year in the US.
- How many fewer new cases of CHD could there be by reducing BMI by 5% in obese adults, compared to reducing tobacco use/exposure by 50%?
- Use evidence from the graph to suggest which intervention the US public should be encouraged to carry out and why.
- i) Describe the trend shown on the graph between reducing salt intake and the number of new cases of CHD per year.
ii) Explain this trend.

Practice Questions — Fact Recall

- Q1 What is an atheroma?
- Q2 Give two effects an atheroma has on the artery it's in.
- Q3 a) Explain how high blood pressure leads to an increased risk of cardiovascular disease.
b) Give three things that can cause an increase in blood pressure.
- Q4 a) Give two examples of risk factors for cardiovascular disease that can be controlled.
b) Give an example of a risk factor for cardiovascular disease that can't be controlled.

6. Transport in Plants — Xylem

Plants are pretty clever when it comes to transporting water. They can take it up from their roots to their leaves against the force of gravity. Let's see how they manage that...

Types of tissue involved in mass transport in plants

- **Xylem** tissue transports water and mineral ions in solution. These substances move up the plant from the roots to the leaves.
- **Phloem** tissue transports organic substances like sugars (also in solution) both up and down the plant — there's more about the phloem on pages 195-198.

Xylem and phloem are mass transport systems (see page 141) — they move substances over large distances.

Learning Objectives:

- Know that xylem is the tissue that transports water in the stem and leaves of plants.
- Understand the cohesion-tension theory of water transport in the xylem.
- Be able to dissect an organ within a plant's mass transport system (Required Practical 5).

Specification Reference 3.3.4.1 and 3.3.4.2

Structure of the xylem

Xylem vessels are the part of the xylem tissue that actually transports the water and ions. Xylem vessels are very long, tube-like structures formed from dead cells (vessel elements) joined end to end. There are no end walls on these cells, making an uninterrupted tube that allows water to pass up through the middle easily.

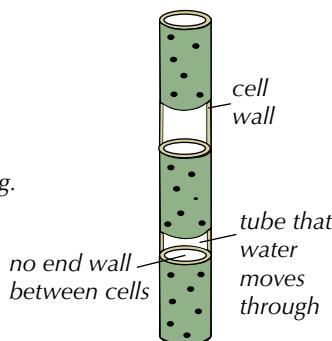


Figure 1: A xylem vessel with internal detail showing.

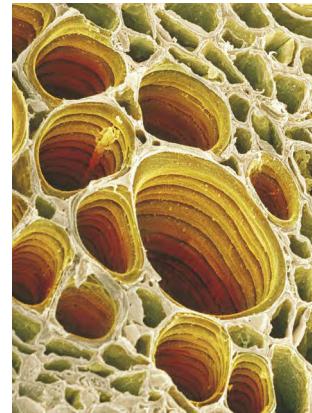


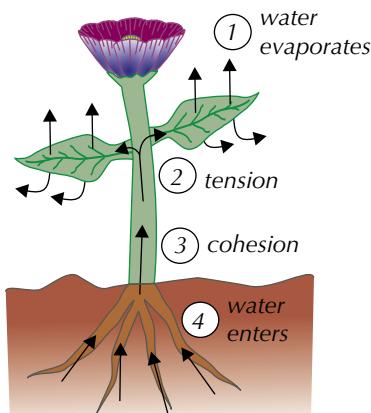
Figure 2: An SEM image of xylem vessels.

Water movement up a plant

Water moves up a plant against the force of gravity, from roots to leaves. This can be explained by the combined action of cohesion and tension.

Cohesion and tension

1. Water evaporates from the leaves at the 'top' of the xylem. This is a process called **transpiration** (see next page).
2. This creates tension (suction), which pulls more water into the leaf.
3. Water molecules are cohesive (they stick together, see page 65) so when some are pulled into the leaf others follow. This means the whole column of water in the xylem, from the leaves down to the roots, moves upwards.
4. Water then enters the stem through the roots.



Tip: This is called the cohesion-tension theory of water transport.

Figure 3: Water movement up a plant.

Tip: Water movement up a plant increases as the transpiration rate increases — see next page.

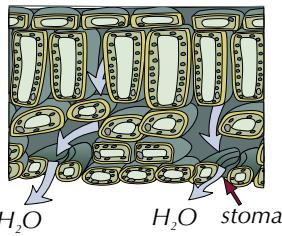


Figure 4: Cross-section of a leaf showing how water moves out during transpiration.

Tip: Transpiration's really a side effect of photosynthesis — the plant needs to open its stomata to let in CO_2 so that it can produce glucose, but this also lets water out.

Tip: Transpiration rate isn't exactly the same as water uptake by a plant — some water is used in reactions (e.g. in photosynthesis) and to support the plant, and some water is produced during respiration.

Tip: You can use a potometer to test the effect of different factors on transpiration rate, e.g. by using a fan to increase air movement or a lamp to increase light intensity, etc.

Tip: If you want to compare water loss from different types of plant, you need to measure the surface area of the leaves because it will vary with type of plant.

Transpiration

Transpiration is the **evaporation** of water from a plant's surface, especially the leaves. Water evaporates from the moist cell walls and accumulates in the spaces between cells in the leaf. When the stomata open (see page 145), it moves out of the leaf down the **water potential gradient** (because there's more water inside the leaf than in the air outside).

Factors affecting transpiration rate

There are four main factors that affect transpiration rate.

1. **Light intensity** — the lighter it is the faster the transpiration rate (i.e. there's a positive correlation between light intensity and transpiration rate). This is because the stomata open when it gets light to let in CO_2 for photosynthesis. When it's dark the stomata are usually closed, so there's little transpiration.
2. **Temperature** — the higher the temperature the faster the transpiration rate. Warmer water molecules have more energy so they evaporate from the cells inside the leaf faster. This increases the water potential gradient between the inside and outside of the leaf, making water diffuse out of the leaf faster.
3. **Humidity** — the lower the humidity, the faster the transpiration rate (i.e. there's a negative correlation between humidity and transpiration rate). If the air around the plant is dry, the water potential gradient between the leaf and the air is increased, which increases transpiration rate.
4. **Wind** — the windier it is, the faster the transpiration rate. Lots of air movement blows away water molecules from around the stomata. This increases the water potential gradient, which increases the rate of transpiration.

Estimating transpiration rate — potometers

A potometer is a special piece of apparatus used to estimate transpiration rates. It actually measures water uptake by a plant, but it's assumed that water uptake by the plant is directly related to water loss by the leaves. You can use it to estimate how different factors affect the transpiration rate.

Here's what you'd do:

1. Cut a shoot underwater to prevent air from entering the xylem. Cut it at a slant to increase the surface area available for water uptake.
2. Assemble the potometer under the water and insert the shoot with the apparatus still under the water, so no air can enter.
3. Remove the apparatus from the water but keep the end of the capillary tube submerged in a beaker of water.
4. Check that the apparatus is watertight and airtight.

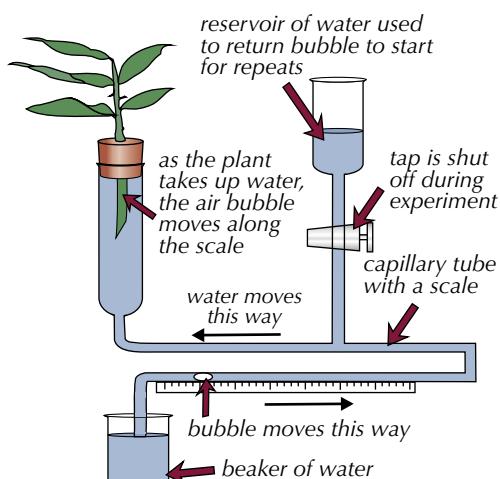


Figure 5: Diagram showing how to use a potometer.

- Dry the leaves, allow time for the shoot to acclimatise and then shut the tap.
- Remove the end of the capillary tube from the beaker of water until one air bubble has formed, then put the end of the tube back into the water.
- Record the starting position of the air bubble.
- Start a stopwatch and record the distance moved by the bubble per unit time, e.g. per hour. The rate of air bubble movement is an estimate of the transpiration rate.
- Remember, only change one variable (e.g. temperature) at a time. All other conditions (e.g. light intensity, humidity) must be kept constant.

Tip: The air bubble is sometimes called the air-water meniscus.

Practice Questions — Application

A potometer was used to test the effect of temperature on transpiration rate. The test was repeated 3 times. The results are shown in the table.

| Temperature (°C) | Distance moved by the bubble in 10 minutes (mm) | | |
|---------------------|---|--------|--------|
| | Test 1 | Test 2 | Test 3 |
| 10 | 15 | 12 | 14 |
| 20 | 19 | 16 | 19 |
| 30 | 25 | 22 | 23 |

- Q1 a) Calculate the mean result for each temperature.
 b) Plot a graph of the mean results and use it to estimate the distance the bubble would move in ten minutes at 25 °C.
 Q2 Describe and explain the results of the experiment.

Tip: To work out the rate of water uptake in mm³ per minute, you need to measure the distance moved by the bubble per minute and the diameter of the capillary tube.

Exam Tip

Repeats are done to increase precision and help to identify anomalous results.

Tip: For data where the distance moved by the bubble is measured per minute or so, a distance-time graph can be plotted. Then the gradient of the line shows the rate of water uptake.

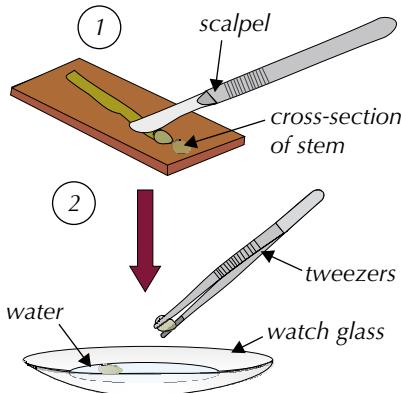
Plant mass transport dissection

You can look at xylem or phloem in plant tissue (e.g. part of a plant stem) under a microscope, and then draw them. But first you need to dissect the plant and prepare a section of the tissue. You can do this using the following method:

REQUIRED PRACTICAL 5

— Example — Looking at the xylem and phloem in a stem —

- Use a scalpel (or razor blade) to cut a cross-section of the stem. Cut the sections as thinly as possible — thin sections are better for viewing under a microscope.
- Use tweezers to gently place the cut sections in water until you come to use them. This stops them from drying out.



Tip: As with all practicals you do, make sure you have carried out a risk assessment before you begin. Pay particular attention to safety when working with sharp blades and remember you need to wear gloves and eye protection when working with stains.

Tip: You could also dissect part of a leaf or root to look at the xylem and phloem there — the basic method of dissection and preparation is the same.

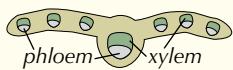
Tip: Lignin is a woody substance which helps to support the walls of xylem cells.

Tip: You can use different stains to highlight different parts of the cells. For example, staining with phloroglucinol will turn the lignin in the xylem red and staining with aniline blue will turn the nucleus blue and the cell walls yellow-brown.

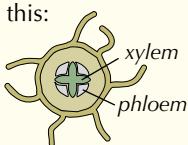


Figure 6: Light micrograph through a stem showing xylem and phloem tissue.

Tip: If you've dissected part of a leaf, the arrangement of xylem and phloem in a cross-section should look something like this:



And if you've dissected part of a root the arrangement should be like this:



3. Add a drop of water to a microscope slide, add the plant section and carefully add one or two drops of a stain, e.g. toluidine blue O (TBO), and leave for about one minute.
4. Carefully apply a cover slip so you have created a temporary mount (see page 83).
5. When you view the specimen under the microscope, if you've used TBO you should be able to see the xylem cells stained blue-green. The phloem cells and the rest of the tissue should appear pinkish purple.

The arrangement of the xylem and the phloem in a cross-section of a stem (of a non-woody plant) is shown in Figure 7. This should help you understand what you are seeing when you look at your stem section under an optical microscope (see Figure 6).

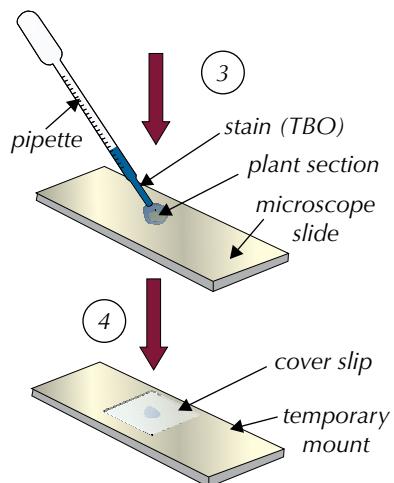


Figure 5: Preparing a cross section of a plant stem for viewing under a microscope.

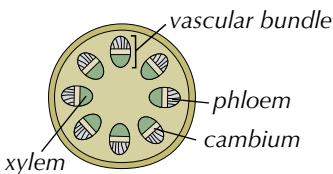


Figure 7: Cross-section of a non-woody stem.

Practice Questions — Fact Recall

- Q1 Which type of plant tissue transports water up the plant from the roots to the leaves?
- Q2 Describe and explain how water can move up a plant.
- Q3 a) Explain how wind affects transpiration rate.
b) Give three other factors that affect the rate of transpiration.
- Q4 Briefly describe how you could prepare a temporary mount of a stem cross-section for observation of the plant's mass transport systems.

7. Transport in Plants — Phloem

The phloem transports dissolved substances around the plant to where they are needed. Scientists still aren't sure exactly how this movement works, but they do have a hypothesis...

Structure and function of the phloem

Solutes are dissolved substances.

Phloem tissue transports organic solutes (mainly sugars like sucrose) round plants. Like xylem, phloem is formed from cells arranged in tubes. Sieve tube elements and companion cells are important cell types in phloem tissue (see Figure 1):

- Sieve tube elements are living cells that form the tube for transporting solutes. They have no nucleus and few organelles, so...
- ...there's a companion cell for each sieve tube element. They carry out living functions for sieve cells, e.g. providing the energy needed for the active transport of solutes.

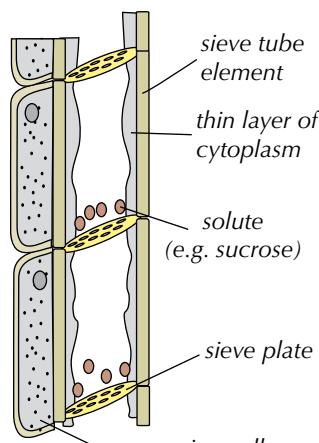


Figure 1: Phloem tissue.

Learning Objectives:

- Know that phloem is the tissue that transports organic substances in plants.
- Know the mass flow hypothesis for the mechanism of translocation in plants.
- Know that ringing and tracer experiments are used to investigate transport in plants.
- Be able to interpret evidence from ringing and tracer experiments, and to evaluate the evidence for and against the mass flow hypothesis.
- Be able to recognise correlations and causal relationships.

Specification Reference 3.3.4.2

What is translocation?

Translocation is the movement of solutes (e.g. amino acids and sugars like sucrose) to where they're needed in a plant. Solutes are sometimes called **assimilates**. It's an energy-requiring process that happens in the phloem.

Translocation moves solutes from 'sources' to 'sinks'. The **source** is where assimilates are produced (so they're at a high concentration there). The **sink** is where assimilates are used up (so they're at a lower concentration there).

Example

The source for sucrose is usually the leaves (where it's made), and the sinks are the other parts of the plant, especially the food storage organs and the meristems (areas of growth) in the roots, stems and leaves.

Enzymes maintain a concentration gradient from the source to the sink by changing the solutes at the sink (e.g. by breaking them down or making them into something else). This makes sure there's always a lower concentration at the sink than at the source.

Example

In potatoes, sucrose is converted to starch in the sink areas, so there's always a lower concentration of sucrose at the sink than inside the phloem. This makes sure a constant supply of new sucrose reaches the sink from the phloem.



Figure 2: Phloem vessels in a Cucurbita plant. The sieve cells are stained blue and the sieve plates are dark green.

Tip: Assimilates are substances that become incorporated into the plant tissue.

Exam Tip

Make sure you learn what the terms 'source' and 'sink' mean — you could be tested on them in the exam.

Tip: There's more about active transport on page 110.

Tip: Experiments have shown that some sucrose is transported also through the cell walls of the phloem.

Tip: Companion cells contain many mitochondria, which means they can make lots of ATP. ATP is needed to actively load the solutes into the phloem at the source.

Tip: There's more about sieve plates and companion cells on the previous page.

The mass flow hypothesis

Scientists still aren't certain exactly how the solutes are transported from source to sink by translocation. The best supported theory is the mass flow hypothesis (see Figure 3):

1. Source

Active transport is used to actively load the solutes (e.g. sucrose from photosynthesis) from companion cells into the sieve tubes of the phloem at the source (e.g. the leaves). This lowers the water potential inside the sieve tubes, so water enters the tubes by osmosis from the xylem and companion cells. This creates a high pressure inside the sieve tubes at the source end of the phloem.

2. Sink

At the sink end, solutes are removed from the phloem to be used up. This increases the water potential inside the sieve tubes, so water also leaves the tubes by osmosis. This lowers the pressure inside the sieve tubes.

3. Flow

The result is a pressure gradient from the source end to the sink end. This gradient pushes solutes along the sieve tubes towards the sink. When they reach the sink the solutes will be used (e.g. in respiration) or stored (e.g. as starch).

The higher the concentration of sucrose at the source, the higher the rate of translocation.

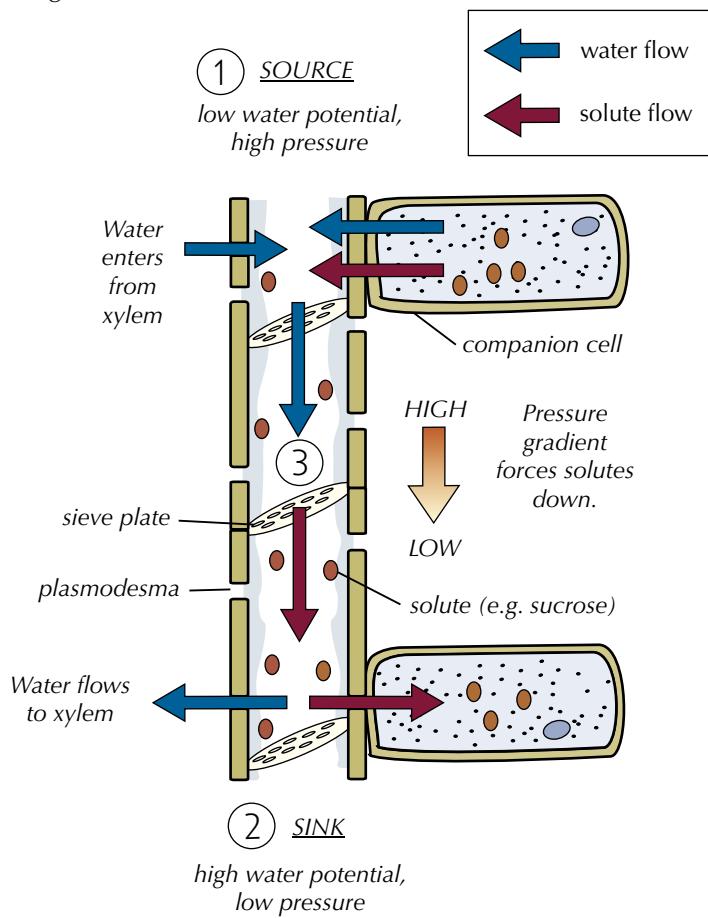


Figure 3: How the mass flow hypothesis works.

Mass flow evidence

There is evidence both for and against mass flow.

Supporting evidence

1. If a ring of bark (which includes the phloem, but not the xylem) is removed from a woody stem, a bulge forms above the ring — see Figure 4. The fluid from the bulge has a higher concentration of sugars than the fluid from below the ring. This is because the sugars can't move past the area where the bark has been removed — this is evidence that there can be a downward flow of sugars.
2. Pressure in the phloem can be investigated using aphids (they pierce the phloem, then their bodies are removed leaving the mouthparts behind, which allows the sap to flow out... gruesome). The sap flows out quicker nearer the leaves than further down the stem — this is evidence that there's a pressure gradient.
3. A **radioactive tracer** such as radioactive carbon (^{14}C) can be used to track the movement of organic substances in a plant (see below).
4. If a metabolic inhibitor (which stops ATP production) is put into the phloem, then translocation stops — this is evidence that active transport is involved.

Objections

1. Sugar travels to many different sinks, not just to the one with the highest water potential, as the model would suggest.
2. The sieve plates would create a barrier to mass flow. A lot of pressure would be needed for the solutes to get through at a reasonable rate.

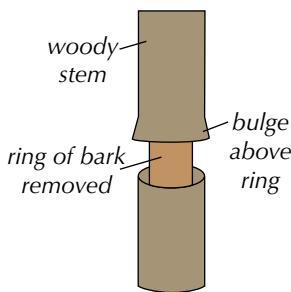


Figure 4: Diagram to show the effect of removing a ring of bark from a tree.

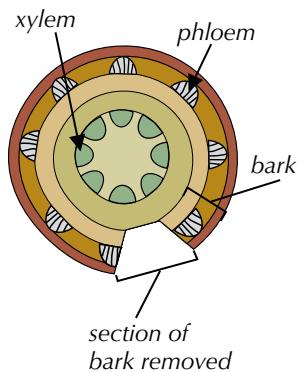


Figure 5: Diagram to show how the removal of bark removes the phloem but leaves the xylem intact.

Tip: Sugars are made in the leaves, so that's why there's a downward flow of sugars (from source to sink) in this case.

Tip: The build up of sugars above the ring causes a decrease in water potential, so water moves into the cells — adding to the bulge.

Exam Tip

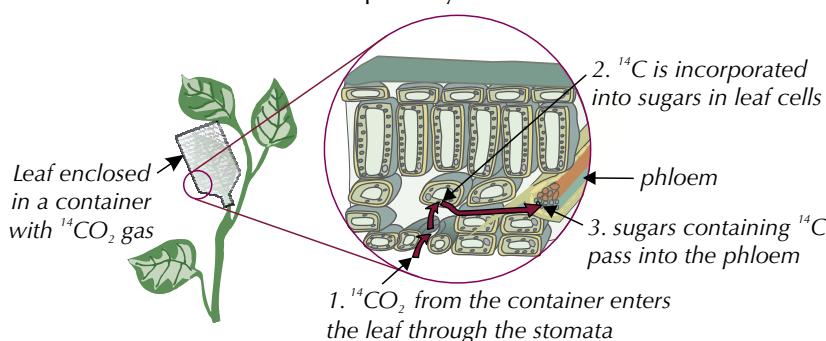
You need to be able to 'evaluate' the evidence — so make sure you really know the evidence for and against the mass flow hypothesis.

Evidence from radioactive tracers

Translocation of solutes can be modelled in an experiment using radioactive tracers. This can be done by supplying part of a plant (often a leaf) with an organic substance that has a radioactive label, then tracking its movement.

Example

Carbon dioxide containing the radioactive isotope ^{14}C is used as a radioactive tracer. This radioactively-labelled CO_2 can be supplied to a single leaf by being pumped into a container which completely surrounds the leaf. The radioactive carbon will then be incorporated into organic substances produced by the leaf (e.g. sugars produced by photosynthesis), which will be moved around the plant by translocation.



Tip: Photosynthesis produces glucose. This is converted to sucrose for transport around the plant.



Figure 6: An autoradiogram showing the fuzzy imprint of a leaf after being given radioactive phosphorus. The black areas show where the radioactive substance is present.

Tip: Looking for correlation from tables is not quite as easy as from graphs but don't panic — if one variable goes up as the other goes up it's a positive correlation. If one variable goes up as the other goes down, it's a negative correlation.

Tip: Correlation and causal relationships come up in lots of topics but the general principles are the same — a correlation between two variables doesn't necessarily mean that one caused the other (see page 15 for more).

The movement of these substances can be tracked using a technique called **autoradiography**. To reveal where the radioactive tracer has spread to in a plant, the plant is killed (e.g. by freezing it using liquid nitrogen) and then the whole plant (or sections of it) is placed onto photographic film — wherever the film turns black, the radioactive substance is present (see Figure 6).

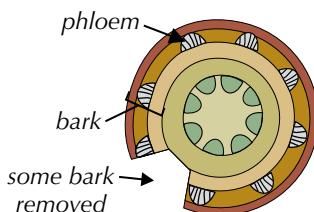
The results demonstrate the translocation of substances from source to sink over time — for example, autoradiographs of plants killed at different times show an overall movement of solutes (e.g. products of photosynthesis) from the leaves towards the roots.

Correlation and causal relationships

The data from experiments used to provide evidence for and against mass flow has to be interpreted carefully.

Example

Scientists carried out a ringing experiment on a particular species of woody plant. A varying amount of bark was left connecting the upper and lower parts of the stems (see below). The plants were left for 24 hours, then the amount of carbohydrate in the plant below the ringing was measured.



| Width of bark strip remaining (% of intact stem) | Carbohydrate transported to the lower part of the stem in 24 hours / mg |
|--|---|
| 0 | 0 |
| 10 | 437 |
| 33 | 609 |
| 87 | 744 |

Correlation

The results in the table above show a positive correlation — as the width of the bark strip remaining increased, the amount of carbohydrate transported to the lower part of the stem (i.e. below the ringing) also increased.

Conclusions

From the results, you might conclude that removing the bark **caused** a reduction in the amount of carbohydrate transported down the stem. This may be because removing more bark, removes more phloem, which reduces the amount of carbohydrate that can be transported. This provides evidence in support of the mass flow hypothesis because the phloem is transporting carbohydrates down from a source in the leaves to a sink in the roots.

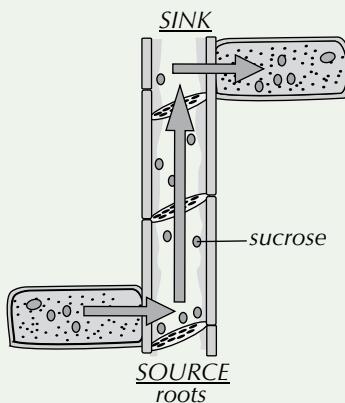
However, you have to be careful when drawing conclusions, especially when there's not much data. The results don't prove that there is a downward flow of sugars in the phloem — there could be other factors affecting the results. For example, it could be that the sugars are actually transported in the xylem, but the xylem tissue was accidentally damaged when the bark was removed. The experiment has also been carried out on only one species of plant, so you can't conclude that this would be the case for all plant species.

However, so many studies have now been done on mass flow, that the correlation shown by this experiment is accepted to be a causal relationship, i.e. removing more of a plant's phloem causes less carbohydrate to be transported downwards, towards a plant's roots.

Practice Questions — Application

Q1 The diagram on the right shows the translocation of sucrose from the roots (where it was stored as starch) to a sink. This process happens in the spring.

- Suggest a sink for the sucrose shown on the diagram.
- Using the mass flow hypothesis:
 - explain why water enters the sieve tubes in the roots,
 - explain why water leaves the sieve tubes at the sink.



Q2 An experiment was done to investigate translocation in a plant using radioactive carbon dioxide ($^{14}\text{CO}_2$). The diagram on the right represents the autoradiography image produced after the experiment. Discuss, using evidence from the diagram, whether or not the experiment provides evidence to support the mass flow hypothesis.



Tip: Remember, radioactive substances show up black during autoradiography.

Practice Questions — Fact Recall

- What substances are transported by the phloem?
- Define translocation.
- What is the difference between a source and a sink in a plant?
- Describe one piece of evidence from ringing experiments that supports the mass flow hypothesis.
- Describe one piece of evidence against the mass flow hypothesis.

Section Summary

Make sure you know...

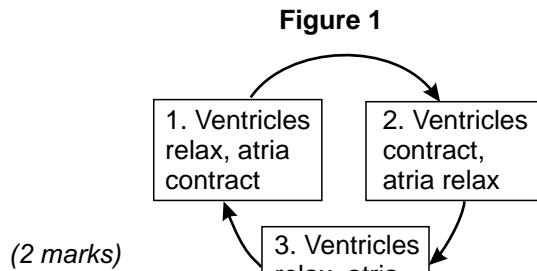
- That during digestion, large molecules, e.g. starch, proteins and lipids, are broken down into smaller molecules by hydrolysis reactions, so that they can be absorbed into the bloodstream.
- That amylase catalyses the breakdown of starch (a polysaccharide).
- That membrane-bound disaccharidases found in cells lining the ileum catalyse the breakdown of disaccharides.
- That lipases catalyse the breakdown of lipids into monoglycerides and fatty acids.
- That bile salts emulsify lipids, increasing the surface area for digestion, and lead to the formation of micelles.
- That peptidases (proteases) catalyse the breakdown of proteins into amino acids.
- That endopeptidases hydrolyse peptide bonds within a protein and exopeptidases hydrolyse bonds at the ends of proteins, removing single amino acids. Membrane-bound dipeptidases are a type of exopeptidase that break down dipeptides into single amino acids.

- That co-transport mechanisms are required for the absorption of monosaccharides, such as glucose.
- The role of micelles in the absorption of lipids, helping to move monoglycerides and fatty acids towards the epithelium, where they can diffuse across the membrane.
- That co-transport mechanisms are required for the absorption of amino acids into the bloodstream.
- That the haemoglobins are a group of chemically similar proteins with a quaternary structure and that they are found in red blood cells, where they are involved in the transport of oxygen.
- That haemoglobin's affinity for oxygen depends on the partial pressure of oxygen (pO_2), loading at high pO_2 and unloading at low pO_2 .
- That the saturation of haemoglobin at any partial pressure is shown on an oxyhaemoglobin dissociation curve.
- That the shape of haemoglobin changes once the first oxygen molecule has joined, making it easier for further oxygen molecules to bind.
- That carbon dioxide concentration also affects the dissociation of oxyhaemoglobin, making it dissociate more easily at higher partial pressures of CO_2 , which is known as the Bohr effect.
- That different organisms are adapted to their environments by having different types of haemoglobin, which vary in their ability to transport oxygen.
- The general pattern of blood circulation in a mammal, including the names of the coronary arteries and of the blood vessels entering and leaving the heart, lungs and kidneys.
- The structure of arteries, arterioles and veins and how they are related to their functions.
- The structure of capillaries and how capillary beds (networks of capillaries) are adapted for efficient diffusion.
- That hydrostatic pressure at the arteriole end of a capillary bed leads to the formation of tissue fluid and that excess tissue fluid is drained into the lymphatic system for return to the circulatory system.
- The internal structure of the heart — vena cava, pulmonary artery, aorta, pulmonary vein, right atrium, left atrium, semi-lunar valves, atrioventricular valves, cords, right ventricle and left ventricle.
- That the right side of the heart pumps deoxygenated blood and the left side pumps oxygenated blood.
- How to safely dissect a mammalian heart.
- The cardiac cycle, including pressure changes, volume changes and valve movements (which maintain a unidirectional flow of blood).
- How to analyse and interpret data relating to pressure and volume changes during the cardiac cycle.
- That cardiovascular diseases affect the heart and blood vessels and usually involve atheromas, which may lead to aneurysms, thrombosis and myocardial infarction (heart attack).
- How to analyse and interpret data associated with specific risk factors (e.g. high blood pressure, high cholesterol, poor diet and smoking), and the incidence of cardiovascular disease, and how to recognise correlations and causal relationships in any data that is given.
- How to evaluate conflicting evidence associated with risk factors affecting cardiovascular disease.
- That xylem vessels transport water round the plant, i.e. up the roots and stem and to the leaves.
- How cohesion and tension move water up the xylem and how transpiration affects this movement.
- How to dissect an organ within a plant's mass transport system.
- That phloem tissue transports dissolved organic substances, such as sucrose, around the plant.
- That the mass flow hypothesis is a theory that explains translocation in a plant — it involves the creation of a pressure gradient, which pushes solutes from source to sink.
- How ringing experiments (which remove a ring of bark from a woody stem) and radioactive tracer experiments are used to investigate transport in plants.
- How to interpret evidence from ringing and tracer experiments, evaluate the evidence for and against the mass flow hypothesis and recognise correlations and causal relationships in the data given.

Exam-style Questions

- 1 **Figure 1** shows the three main stages of the cardiac cycle.

- 1.1 Explain the pressure change that occurs in the atria in the first stage of the cardiac cycle.



- 1.2 Name the valves that connect the atria to the ventricles and describe their function.

(2 marks)

- 1.3 During stages one and two, are the valves connecting the atria to the ventricles open or closed? Explain your answer(s).

(2 marks)

- 1.4 During stage three, why does the pressure of the atria increase?

(1 mark)

- 1.5 Name the vessel in which the blood leaves the right ventricle to travel to the lungs.

(1 mark)

- 1.6 Suggest how the structure of this vessel is related to its function.

(3 marks)

- 2 A student used a potometer to investigate the effect of light intensity on transpiration rate. Her results are shown in **Figure 2**.

- 2.1 Using the graph, work out the rate of bubble movement for a light intensity of **1.5 arbitrary units**. Give your answer in mm min^{-1} .

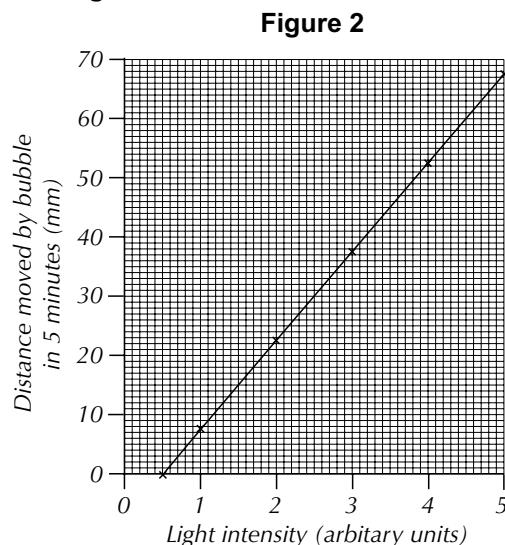
(2 marks)

- 2.2 Using the cohesion-tension theory, explain the results shown by the graph.

(4 marks)

- 2.3 Suggest what **negative control** should be used for this investigation.

(1 mark)



3 A scientist was investigating the link between poor diet and cardiovascular disease. He took 1000 British men, aged 40-60 years old and put them into two groups. One group was given dietary information on how to reduce their risk of cardiovascular disease, e.g. by lowering their saturated fat intake. The other group wasn't given any information. The scientist recorded any deaths from cardiovascular disease over ten years.

- 3.1** What is cardiovascular disease? (1 mark)
- 3.2** What is the name given to the group of men that wasn't given any information? (1 mark)
- 3.3** Give **two** ways in which the study could have been improved. (2 marks)
- 3.4** It's important to have some fat in the diet to stay healthy.
Explain how dietary fats are digested and absorbed onto the bloodstream. (3 marks)

4 Cats, pumas and foxes were used in a study to investigate haemoglobin's affinity for oxygen. For each type of animal, blood was taken from a sample that lived at sea level and a sample that lived at high altitude. The pO_2 at which each animal's haemoglobin was 50% saturated was recorded. The results are shown in **Table 1**.

Table 1

| Animal | Sea Level (pO_2 / kPa) | High Altitude (pO_2 / kPa) |
|--------|------------------------------|----------------------------------|
| Cat | 3.9 | 3.0 |
| Puma | 4.8 | 4.1 |
| Fox | 3.5 | 2.5 |

- 4.1** Describe the structure of haemoglobin. (2 marks)
- 4.2** There is less oxygen at high altitudes than at sea level.
Use evidence from **Table 1** to support this statement. (3 marks)

5 A fruit grower cut a C-shaped ring in the bark of the trunks of his fruit trees. He did this so that the branches would receive more nutrients.

- 5.1** What type of plant tissue involved in mass transport was removed with the bark? (1 mark)
- 5.2** Describe how this tissue is involved in mass transport in plants. (2 marks)
- 5.3** Explain how the fruit grower's method may result in the trees producing more fruit. (3 marks)

1. DNA

DNA is stored differently in prokaryotic and eukaryotic cells.

How is DNA stored?

Although the structure of DNA is the same in all organisms, eukaryotic and prokaryotic cells store DNA in slightly different ways. (For a recap on the differences between prokaryotic and eukaryotic cells see pages 70 and 77.)

Eukaryotic cells

Eukaryotic cells contain linear DNA molecules that exist as chromosomes — thread-like structures, each made up of one long molecule of DNA and its associated proteins. Chromosomes are found in the nucleus.

The DNA molecule is really long, so it has to be wound up so it can fit into the nucleus. It's wound around proteins called **histones**. Histone proteins also help to support the DNA. The DNA (and protein) is then coiled up very tightly to make a compact chromosome.

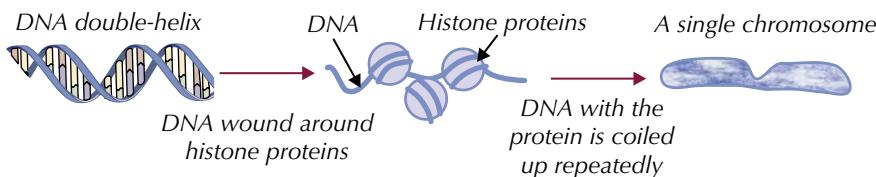


Figure 1: Storage of DNA in eukaryotes.

The mitochondria and chloroplasts in eukaryotic cells also have their own DNA. This is pretty similar to prokaryotic DNA (see below) because it's circular and shorter than DNA molecules in the nucleus. It's not associated with histone proteins.

Prokaryotic cells

Prokaryotes also carry DNA as chromosomes — but the DNA molecules are shorter and circular. The DNA isn't wound around histones — it condenses to fit in the cell by supercoiling.

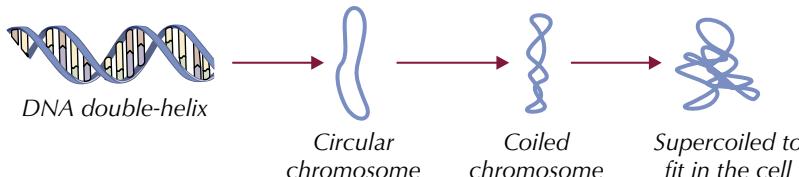


Figure 2: Storage of DNA in prokaryotes.

Learning Objectives:

- Know that in the nucleus of eukaryotic cells, DNA molecules are very long, linear and associated with proteins, called histones.
- Know that in eukaryotic cells, a DNA molecule together with its associated proteins form a chromosome.
- Know that the mitochondria and chloroplasts of eukaryotic cells also contain DNA, which, like the DNA of prokaryotes, is short, circular and not associated with proteins.
- Know that in prokaryotic cells, DNA molecules are short, circular and not associated with proteins.

Specification Reference 3.4.1

Tip: Eukaryotic cells include animal and plant cells. Prokaryotic cells are generally bacteria.

Practice Questions — Fact Recall

Q1 Describe the structure of a eukaryotic chromosome.

Q2 Describe how DNA in prokaryotic chromosomes differs from DNA in eukaryotic chromosomes.

Learning Objectives:

- Know that a gene is a base sequence of DNA that codes for the amino acid sequence of a polypeptide or a functional RNA (including ribosomal RNA and tRNAs).
- Know that a sequence of three DNA bases, called a triplet, codes for a specific amino acid.
- Understand the concept of the genome as the complete set of genes in a cell and of the proteome as the full range of proteins that a cell is able to produce.
- Know that in eukaryotes, much of the nuclear DNA does not code for polypeptides. There are, for example, non-coding multiple repeats of base sequences between genes. Even within a gene only some sequences, called exons, code for amino acid sequences. Within the gene, these exons are separated by one or more non-coding sequences called introns.
- Know that a gene occupies a fixed position, called a locus, on a particular DNA molecule.

Specification
Reference 3.4.1 and 3.4.2

Tip: Actual genes are much, much longer than the one in Figure 1 — thousands of base pairs.

2. Genes and Chromosomes

*Only a small amount of the DNA in a cell carries genetic information.
The most important parts of a DNA molecule are the genes.*

Genes

DNA contains genes. A gene is a sequence of DNA bases (see page 54) that codes for either a polypeptide or functional RNA (see below). The sequence of amino acids in a polypeptide forms the primary structure of a protein (see page 34).

Different polypeptides have a different number and order of amino acids. It's the order of bases in a gene that determines the order of amino acids in a particular polypeptide. Each amino acid is coded for by a sequence of three bases in a gene called a triplet or codon (see page 211). To make a polypeptide, DNA is first copied into messenger RNA (mRNA). This is the first stage of protein synthesis (see page 206).

Genes that don't code for a polypeptide code for **functional RNA** instead. Functional RNA is RNA molecules other than mRNA, which perform special tasks during protein synthesis, e.g. tRNA (see page 206) and ribosomal RNA (rRNA), which forms part of ribosomes.

The complete set of genes in a cell is known as the cell's **genome** and the full range of proteins that the cell is able to produce is known as its **proteome**.

Non-coding DNA

In eukaryotes, a lot of the nuclear DNA (DNA stored in the nucleus) doesn't code for polypeptides. Some genes don't code for polypeptides at all — they code for functional RNA (see above).

Even genes that do code for polypeptides contain sections that don't code for amino acids. These sections of DNA are called **introns**. There can be several introns within a gene and their purpose isn't known for sure. Introns in eukaryotes are removed during protein synthesis — so they don't affect the amino acid order. Prokaryotic DNA doesn't have introns. All the bits of a gene that do code for amino acids are called **exons**.

Eukaryotic DNA also contains regions of multiple repeats outside of genes. These are DNA sequences that repeat over and over. For example: CCTTCCTTCCTT. These areas don't code for amino acids either, so they're called non-coding multiple repeats.

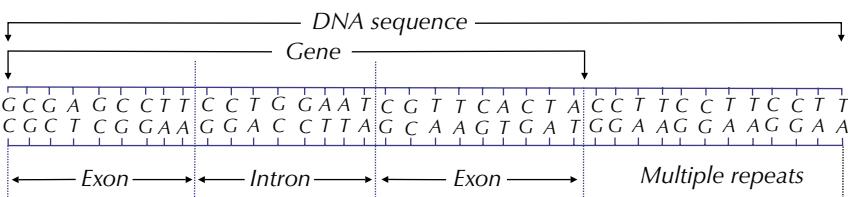


Figure 1: Diagram showing different types of non-coding DNA.

Alleles

A gene can exist in more than one form. These forms are called alleles. The order of bases in each allele is slightly different, so they code for slightly different versions of the same polypeptide.

Example

The gene that codes for blood type exists as one of three alleles — one codes for type O, another for type A and the other for type B.

Homologous chromosomes

In a eukaryotic cell nucleus, DNA is stored as chromosomes. Humans have 23 pairs of chromosomes, 46 in total — two number 1s, two number 2s, two number 3s, etc. Pairs of matching chromosomes (e.g. the 1s) are called **homologous pairs**.

In a homologous pair both chromosomes are the same size and have the same genes, although they could have different alleles. Alleles coding for the same characteristic will be found at the same fixed position (**locus**) on each chromosome in a homologous pair. This is illustrated in Figure 3.

Example

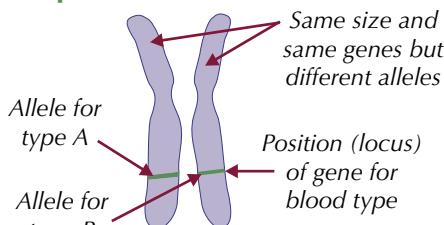


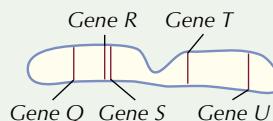
Figure 3: Diagram showing a pair of homologous chromosomes.



Figure 2: A complete set of 46 chromosomes from a human male.

Practice Questions — Application

- Q1 The diagram on the right shows the position of five genes on a chromosome.
Draw the homologous chromosome.



- Q2 The diagram below shows the sequence of a short stretch of DNA:

Gene
ACTGTAT CCTATCGC TGATCGA TGCTCG ATGTCTA GCGCGCGCGC
| Exon | Intron | Exon | Intron | Exon |

- Write down the base sequence that actually determines the order of amino acids in the protein.
- How many bases long is the region of multiple repeats?
- Write down the base sequence that is repeated in the multiple repeat region.

Tip: Don't get confused between introns and exons. Just remember — Introns Interrupt the exons, which code for protein.

Practice Questions — Fact Recall

- What is a gene?
- How many DNA bases code for one amino acid?
- What is a cell's genome?
- What is a cell's proteome?
- Name two types of non-coding DNA.
- Name the sections within genes that code for amino acids.
- What is an allele?
- Alleles for the same characteristic can be found at a particular fixed point on a chromosome. What is the name given to this fixed point?

Learning Objective:

- Know the structure of molecules of messenger RNA (mRNA) and of transfer RNA (tRNA).

Specification Reference 3.4.2

Tip: There's more on transcription and translation on p. 207-210. There's more on ribosomes on p. 74.

Tip: mRNA is copied from DNA — so its sequence is complementary to the DNA sequence. See page 54 for more.

3. RNA and Protein Synthesis

There are two types of RNA that play a key role in protein synthesis.

What is protein synthesis?

Protein synthesis is the production of proteins (polypeptides) from the information contained within a cell's DNA. It's also known as polypeptide synthesis. It involves two main stages:

- Transcription — where the DNA code is copied into a molecule called mRNA (see pages 207-208).
- Translation — where the mRNA joins with an organelle called a ribosome and the code it carries is used to synthesise a protein (see pages 209-210).

RNA

Remember, RNA is a single polynucleotide strand and it contains uracil (U) as a base instead of thymine (see page 55). Uracil always pairs with adenine during protein synthesis. RNA isn't all the same though — there are different types. You need to know about mRNA and tRNA.

Messenger RNA (mRNA)

mRNA is made during transcription. It carries the genetic code from the DNA to the ribosomes, where it's used to make a protein during translation. mRNA is a single polynucleotide strand. In mRNA, groups of three adjacent bases are usually called **codons** (they're sometimes called triplets or base triplets).

Transfer RNA (tRNA)

tRNA is involved in translation. It carries the amino acids that are used to make proteins to the ribosomes. tRNA is a single polynucleotide strand that's folded into a clover shape. Hydrogen bonds between specific base pairs hold the molecule in this shape. Every tRNA molecule has a specific sequence of three bases at one end called an anticodon. It also has an amino acid binding site at the other end.

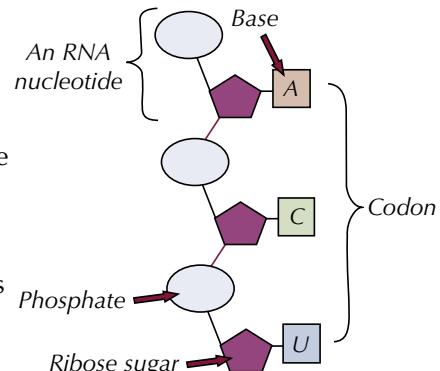


Figure 1: The structure of mRNA.

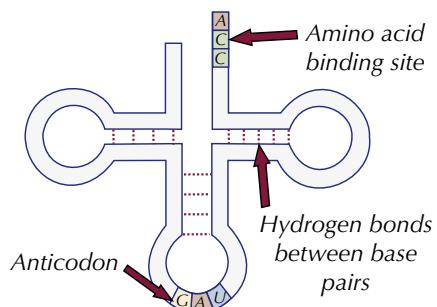


Figure 2: The structure of tRNA.

Practice Questions — Fact Recall

- What role does mRNA play in protein synthesis?
- What is an mRNA codon?
- What does 'mRNA' stand for?
- What does 'tRNA' stand for?

4. Transcription and Translation

Proteins are synthesised (made) using the instructions in DNA.

Protein synthesis involves transcription and translation.

Transcription

During transcription an mRNA copy of a gene is made from DNA. In eukaryotic cells, transcription takes place in the nucleus. Prokaryotes don't have a nucleus, so transcription takes place in the cytoplasm. Here's how transcription happens:

1. RNA polymerase attaches to the DNA

Transcription starts when **RNA polymerase** (an enzyme) attaches to the DNA double-helix at the beginning of a gene.

In eukaryotes, the hydrogen bonds between the two DNA strands in the gene are broken by a DNA helicase attached to the RNA polymerase. This separates the strands, and the DNA molecule uncoils at that point, exposing some of the bases. One of the strands is then used as a template to make an mRNA copy — see Figure 1.

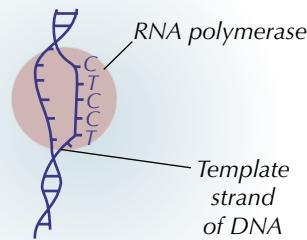


Figure 1: RNA polymerase attaches to the DNA double-helix.

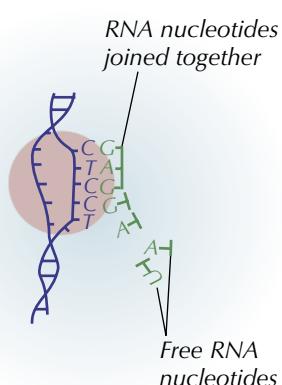


Figure 2: A complementary mRNA strand starts to form.

2. Complementary mRNA is formed

The RNA polymerase lines up free RNA nucleotides alongside the exposed bases on the template strand. The free bases are attracted to the exposed bases. Specific, complementary base pairing (see p. 54) means that the mRNA strand ends up being a **complementary copy** of the DNA template strand (except the base T is replaced by U in RNA). Once the RNA nucleotides have paired up with their specific bases on the DNA strand, they're joined together by RNA polymerase, forming an mRNA strand — see Figure 2.

Learning Objectives:

- Know that transcription is the production of mRNA from DNA.
- Recall the role of RNA polymerase in joining mRNA nucleotides.
- Know that in eukaryotes, transcription results in the production of pre-mRNA. This is then spliced to form mRNA.
- Know that, in prokaryotes, transcription results directly in the production of mRNA from DNA.
- Know that translation is the production of polypeptides from the sequence of codons carried by mRNA.
- Understand the roles of ribosomes, tRNA and ATP in translation.

Specification Reference 3.4.2

Tip: In prokaryotes, the DNA strands are separated by RNA polymerase.

Tip: Free RNA nucleotides aren't attached to anything — they're just floating freely in the nucleus.

3. RNA polymerase moves down the DNA strand

The RNA polymerase moves along the DNA, assembling the mRNA strand. The hydrogen bonds between the uncoiled strands of DNA re-form once the RNA polymerase has passed by and the strands coil back into a double-helix — see Figure 3.

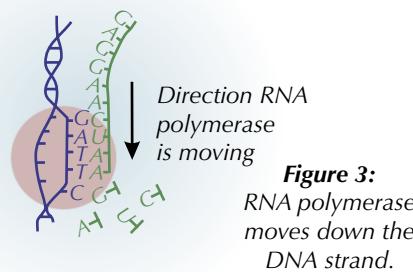


Figure 3:
RNA polymerase moves down the DNA strand.

Tip: Complementary RNA nucleotides bind to each DNA triplet and a complementary mRNA strand forms.

DNA triplet
codon
on mRNA

A T C
U A G

Tip: mRNA acts as a messenger by carrying genetic information between DNA and the ribosomes — that's how it gets its name.

Tip: Stop signals are particular base triplets, see page 211 for more.

4. RNA polymerase reaches stop signal

When RNA polymerase reaches a particular sequence of DNA called a **stop signal**, it stops making mRNA and detaches from the DNA.

In eukaryotes, mRNA moves out of the nucleus through a nuclear pore and attaches to a ribosome in the cytoplasm, where the next stage of protein synthesis takes place (see next page).

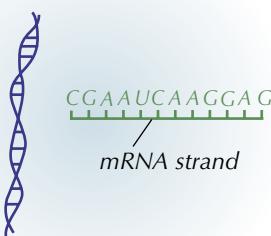


Figure 4: mRNA detaches from the DNA.

Editing mRNA

Tip: Remember, introns are the bits of a gene that don't code for anything and exons are the bits that do — see page 204.

Tip: The pre-mRNA strand shown here is complementary to the DNA template strand that it's been transcribed from:



Transcription produces different products in eukaryotes and prokaryotes. In eukaryotes, the introns and exons are both copied into mRNA during transcription. mRNA strands containing introns and exons are called **pre-mRNA**. A process called **splicing** then occurs — introns are removed and the exons joined together — forming mRNA strands (see Figure 5). This takes place in the nucleus. The mRNA then leaves the nucleus for the next stage of protein synthesis (translation).

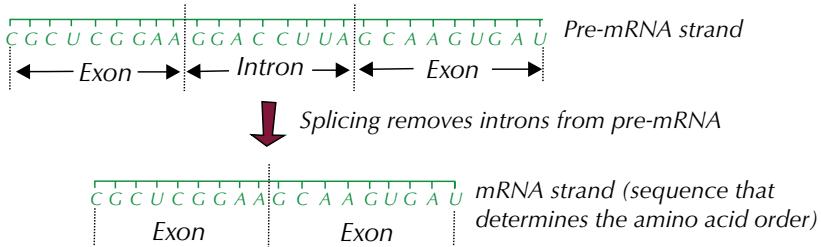


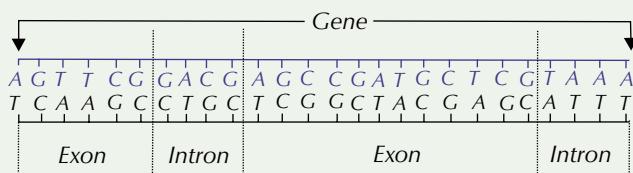
Figure 5: Pre-mRNA is spliced to produce mRNA.

In prokaryotes, mRNA is produced directly from the DNA — without splicing taking place. There's no need for splicing because there are no introns in prokaryotic DNA.

Practice Questions — Application

Q1 α -amanitin is a deadly toxin produced by some mushrooms. It works by inhibiting RNA polymerase. What effect will this have on protein synthesis? Explain your answer.

Q2 Part of the DNA sequence of a gene is shown below.



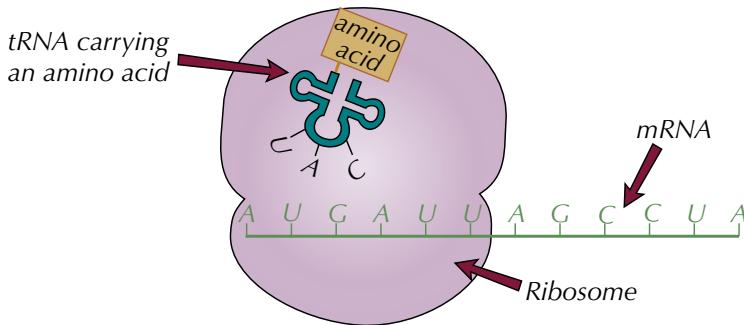
- A molecule of pre-mRNA is transcribed using the blue strand as a template. Write down the sequence of this pre-mRNA molecule.
- The pre-mRNA molecule is spliced to produce mRNA. How many amino acids would this mRNA strand code for?

Translation

Translation is the second stage of protein synthesis. In both eukaryotes and prokaryotes, translation occurs at the ribosomes in the cytoplasm. During translation, amino acids are joined together to make a polypeptide chain (protein), following the sequence of codons carried by the mRNA. Here's how it works:

The mRNA attaches itself to a ribosome and transfer RNA (tRNA) molecules carry amino acids to it. ATP provides the energy needed for the bond between the amino acid and the tRNA molecule to form.

Tip: Proteins are made up of one or more polypeptide chains. Protein synthesis is sometimes called polypeptide synthesis for this reason.



A tRNA molecule (carrying an amino acid), with an anticodon that's complementary to the first codon on the mRNA, attaches itself to the mRNA by complementary base pairing. A second tRNA molecule attaches itself to the next codon on the mRNA in the same way.

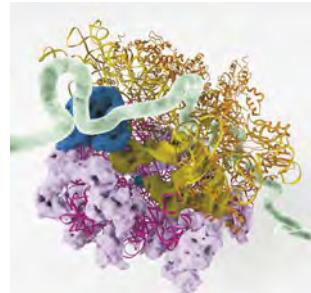
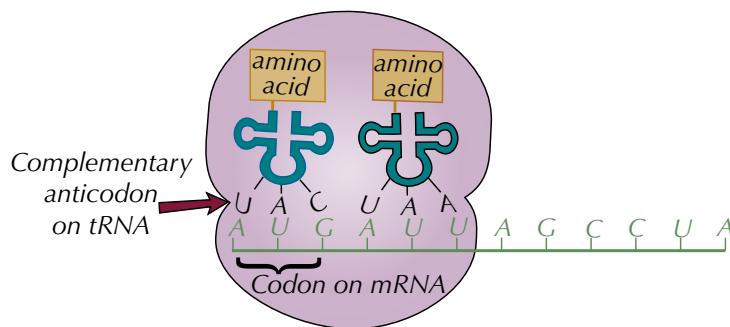
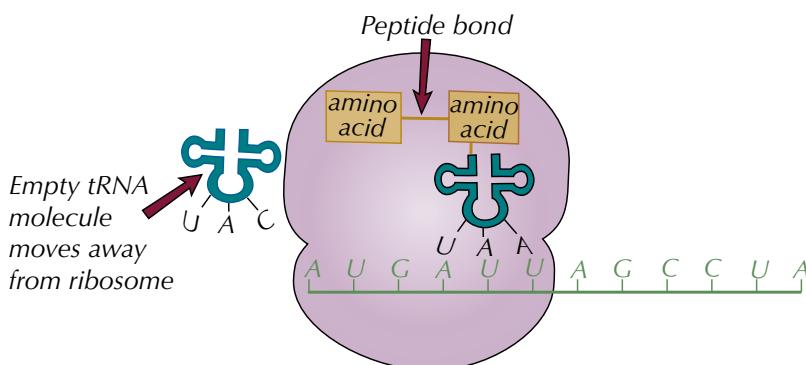
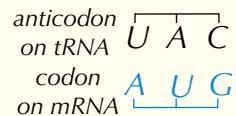


Figure 6: mRNA (turquoise) attached to a bacterial ribosome.



The two amino acids attached to the tRNA molecules are joined by a peptide bond. The first tRNA molecule moves away, leaving its amino acid behind.

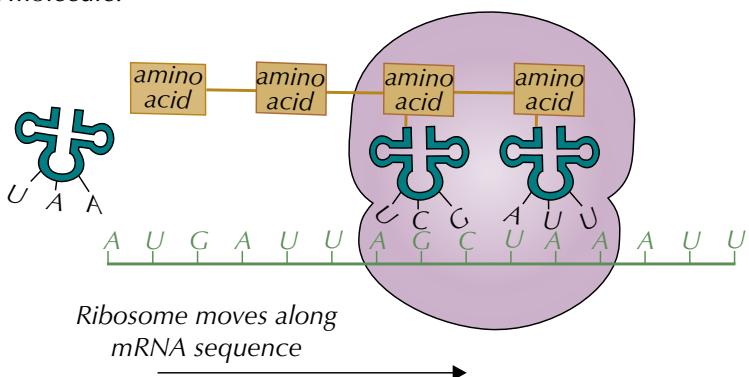
Tip: The tRNA anticodon binds to a complementary codon on the mRNA.



Tip: See page 206 for more on the structure of mRNA and tRNA.

Tip: Once the amino acids are lined up in the correct order, the ribosome joins them together.

A third tRNA molecule binds to the next codon on the mRNA. Its amino acid binds to the first two and the second tRNA molecule moves away. This process continues, producing a chain of linked amino acids (a polypeptide chain), until there's a stop signal (see next page) on the mRNA molecule.



The polypeptide chain (protein) then moves away from the ribosome and translation is complete.

Polypeptide chain → amino acid — amino acid — amino acid — amino acid — ...

Practice Questions — Application

Tip: A mutation is any change to the DNA base sequence. See page 223 for more.

- Q1 Diamond-Blackfan anaemia is an inherited condition caused by one of several gene mutations. The mutations can affect the function of the proteins that make up ribosomes. What effect could this have on protein synthesis? Explain your answer.
- Q2 An error occurs during transcription that accidentally inserts a stop signal into the middle of an mRNA sequence. What effect could this have on the protein that is eventually produced? Explain your answer.

Practice Questions — Fact Recall

Tip: Don't get confused between mRNA and tRNA... Take another look at page 206 if you need a recap.

- Q1 Name the two stages of protein synthesis and state where each one takes place in eukaryotes.
- Q2 a) What is RNA polymerase?
b) In which stage of protein synthesis is it involved?
- Q3 Why is the mRNA that's produced from a DNA template always a complementary copy of the DNA?
- Q4 Explain why eukaryotic mRNA gets spliced.
- Q5 Why does prokaryotic mRNA not undergo splicing?
- Q6 Describe the function of tRNA.
- Q7 What role does ATP play in translation?
- Q8 Explain how tRNA molecules pair up with mRNA during protein synthesis.
- Q9 What type of bond joins two amino acids together?

5. The Genetic Code and Nucleic Acids

The genetic code is pretty important — it encodes the information in genes and these determine what we look like, how we develop and much, much more. Which is probably why the examiners expect you to know all about the genetic code...

What is the genetic code?

The genetic code is the sequence of base triplets (codons) in mRNA which code for specific amino acids. In the genetic code, each base triplet is read in sequence, separate from the triplet before it and after it. Base triplets don't share their bases — the code is **non-overlapping**.

Examples

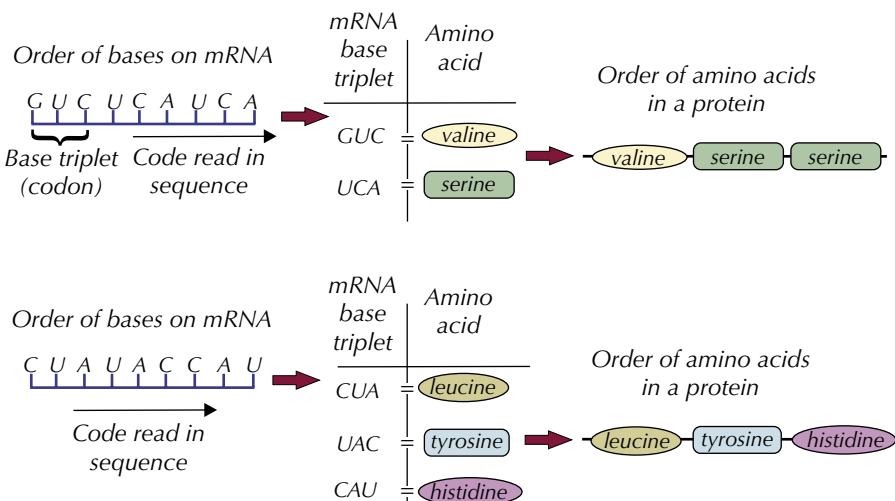


Figure 1: Examples to explain how the non-overlapping genetic code works.

The genetic code is also **degenerate** — there are more possible combinations of triplets than there are amino acids (20 amino acids but 64 possible triplets). This means that some amino acids are coded for by more than one base triplet, e.g. tyrosine can be coded for by UAU or UAC. Not all triplets code for amino acids though. For example, some triplets are used to tell the cell when to stop production of a protein — these are called stop signals. They're found at the end of the mRNA. E.g. UAG is a stop signal. (There are also start signals at the start of the mRNA which tell the cell when to start protein production, but these code for a specific amino acid called methionine.)

The genetic code is also **universal** — the same specific base triplets code for the same amino acids in all living things. E.g. UAU codes for tyrosine in all organisms.

Interpreting data on nucleic acids

You might have to interpret information on nucleic acids in the exam. The examples on the next page show you the sorts of data you might get given and the things you might be asked to do.

Learning Objectives:

- Know that the genetic code is universal, non-overlapping and degenerate.
- Be able to relate the base sequence of nucleic acids to the amino acid sequence of polypeptides, when provided with suitable data about the genetic code.
- Be able to interpret data from experimental work investigating the role of nucleic acids.

Specification Reference 3.4.1 and 3.4.2

Tip: The same genetic code is found in all organisms that are found on Earth. This provides indirect evidence for evolution, as it suggests that the code might have been preserved from a common ancestor of all living organisms.

Exam Tip

You don't need to learn any of these codons or the amino acids they code for — any information you need to answer these questions will be given to you in the exam.

Examples

The mRNA codons for some amino acids are given in the table on the right.

| mRNA codon | Amino acid |
|------------|------------|
| UCU | Serine |
| CUA | Leucine |
| UAU | Tyrosine |
| GUG | Valine |
| GCA | Alanine |
| CGC | Arginine |

Tip: When interpreting data on nucleic acids remember that DNA contains T and RNA contains U.

Tip: You could also be asked to work out the amino acids from a given DNA sequence and a table.

Tip: You might be asked to name the amino acid coded for by a tRNA anticodon using a table like the one at the top of the page.

Tip: You might have to work out the sequence of some mRNA from a sequence of amino acids and a table.

You might be asked to give the DNA sequence for amino acids...

Because mRNA is a complementary copy of the DNA template, the DNA sequence for each amino acid is made up of bases that would pair with the mRNA sequence. The DNA sequence is shown in the table below.

| mRNA codon | Amino acid | DNA sequence |
|------------|------------|--------------|
| UCU | Serine | AGA |
| CUA | Leucine | GAT |
| UAU | Tyrosine | ATA |
| GUG | Valine | CAC |
| GCA | Alanine | CGT |
| CGC | Arginine | GCG |

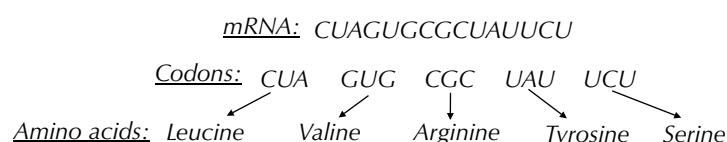
...or to give the tRNA anticodons from mRNA codons...

tRNA anticodons are complementary copies of mRNA codons, so you can work out the tRNA anticodon from the mRNA codon:

| mRNA codon | tRNA anticodon |
|------------|----------------|
| UCU | AGA |
| CUA | GAU |
| UAU | AUA |
| GUG | CAC |
| GCA | CGU |
| CGC | GCG |

...or to write the amino acid sequence for a section of mRNA

To work out the sequence of amino acids from some mRNA, you need to break the genetic code into codons and then use the information in the table to work out what amino acid they code for.



Practice Questions — Application

The table on the right shows some mRNA codons and the amino acids they code for.

- Q1 Using the table, give the mRNA codons for the following amino acid sequence:

Tyr - Phe - Gln - Ile - Ala - His

- Q2 Give the DNA base sequence that would code for the following amino acid sequence:

Met - Phe - Gln - Gln - Ala - Tyr - Ile

| mRNA codon | Amino Acid |
|------------|------------|
| UUU | Phe |
| UAC | Tyr |
| CAA | Gln |
| GCG | Ala |
| AUG | Met |
| CAU | His |
| AUA | Ile |

- Q3 Give the tRNA anticodons for the following mRNA codons:

AUGCAUUAUACAUUUUCAA

- Q4 Write down the amino acid sequence that would be produced from the following tRNA anticodons:

AAAGUUUAUGUACGCAUG

- Q5 The DNA base sequence below codes for the amino acid sequence beneath it. Neither is complete. Fill in the blanks, using the information in the table to help you.

DNA: GTA - __ - __ - AAA - ATG - __ - GTA

Amino acid: His - Ala - Ile - Phe - __ - Gln - __

Tip: The amino acids given in the table are abbreviations — e.g. ‘Phe’ is short for Phenylalanine.

Tip: To answer Q2, don’t jump straight into working out the DNA base sequence. You’ve got to find the complementary mRNA codon from the table first.

Tip: It’s easy to make a mistake if you misread one of the letters in the table, so double-check your answers to make sure you’ve got it right.

Interpreting experimental data on nucleic acids

In the exam you might have to interpret data from experiments done to investigate nucleic acids and their role in protein synthesis.

Example

To investigate how two new drugs affect nucleic acids and their role in protein synthesis, bacteria were grown in normal conditions for a few generations, then moved to media containing the drugs. After a short period of time, the concentration of protein and complete strands of mRNA in the bacteria were analysed. The results are shown in Figure 2.

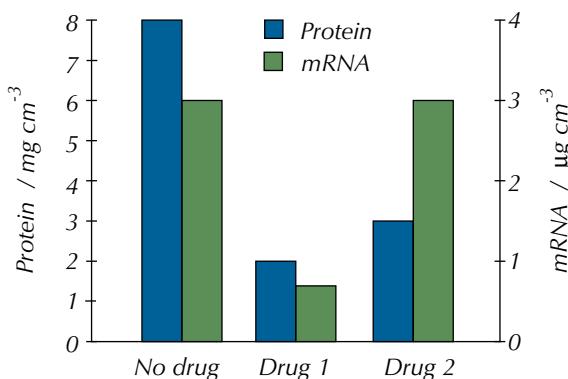


Figure 2: Bar chart to show mRNA and protein concentration in the presence and absence of drugs.

Exam Tip

You don’t need to learn this example — it’s just here to show you the sort of thing you might get in the exam.

Tip: Transcription and translation are on pages 207–210.

Tip: A molecule capable of binding to mRNA would have had a similar effect to drug 1, since it would have prevented mRNA being read by the ribosomes and stopped it being translated.

Tip: Leucine is an amino acid.

Figure 2 on the previous page shows that both mRNA and protein concentration were lower in the presence of drug 1 compared to the no-drug control. This suggests that drug 1 affects the production of full length mRNA, so there's no mRNA for protein synthesis during translation.

mRNA production in the presence of drug 2 was unaffected, but less protein was produced — 3 mg cm⁻³ compared to 8 mg cm⁻³. This suggests that drug 2 interferes with translation. mRNA was produced, but less protein was translated from it.

Further tests to establish the nature of the two drugs were carried out. Drug 1 was found to be a ribonuclease (an enzyme that digests RNA). This could explain the results of the first experiment — most strands of mRNA produced by the cell would be digested by drug 1, so couldn't be used in translation to make proteins.

Drug 2 was found to be a single-stranded, clover-shaped molecule capable of binding to the ribosome. Again, this helps to explain the results from the first experiment — drug 2 could work by binding to the ribosome, blocking tRNAs from binding to it and so preventing translation.

Practice Questions — Application

A chemical called puromycin is believed to affect the development of rapid respiration in a freshly cut potato slice, by either affecting the synthesis of proteins or nucleic acids. In an experiment, potato slices were kept in various concentrations of puromycin for 24 hours. Nucleic acid synthesis was monitored by radioactively tagging uracil and then measuring its uptake. Protein synthesis was monitored by radioactively tagging leucine and then measuring its uptake. Afterwards the percentage inhibition of the development of respiration, leucine uptake and uracil uptake were calculated. The results are shown in the table below.

| Puromycin concentration (mol/l) | % inhibition of the development of respiration | % inhibition of leucine uptake | % inhibition of uracil uptake |
|---------------------------------|--|--------------------------------|-------------------------------|
| Control | - | - | - |
| 0.6×10^{-4} | 33 | 32 | 19 |
| 1.0×10^{-4} | 49 | 55 | 31 |
| 2.0×10^{-4} | 76 | 73 | 55 |
| 4.0×10^{-4} | 97 | 93 | 79 |

- Q1 Suggest why uracil was the only base that was radioactively tagged.
- Q2 Describe the results shown in the table for the four different concentrations of puromycin used.
- Q3 Do the results show that puromycin has a greater effect on nucleic acid synthesis or protein synthesis?

Practice Questions — Fact Recall

- Q1 The genetic code is described as 'non-overlapping'. What does this mean?
- Q2 What is meant by the term 'start signal' in mRNA?
- Q3 The same base triplets code for the same amino acids in all living things. What word is used to describe this feature of the genetic code?

Section Summary

Make sure you know...

- How DNA is stored as chromosomes in eukaryotes and prokaryotes. In eukaryotes, long, linear DNA molecules are wound around proteins called histones and then coil up to form compact chromosomes, which are stored in the nucleus. In prokaryotes, DNA molecules are shorter and circular. They condense by supercoiling and aren't associated with histone proteins.
- That the DNA in mitochondria and chloroplasts in eukaryotic cells is similar to prokaryotic DNA in its structure.
- That genes are base sequences of DNA that code for a polypeptide or a functional RNA.
- That a sequence of three DNA bases, known as a triplet (or codon), codes for one amino acid in a polypeptide.
- That a cell's genome is the complete set of genes in the cell and a cell's proteome is the full range of proteins that the cell is able to produce.
- That much of the nuclear DNA in eukaryotic cells doesn't code for polypeptides. Even within genes that code for polypeptides, only the exons code for amino acids. Two types of non-coding DNA in eukaryotes are introns (within genes) and multiple repeats (between genes).
- That alleles are different versions of the same gene and that alleles coding for the same characteristic are found at the same position (locus) on each chromosome in a homologous pair.
- That mRNA (messenger RNA) is made of a single polynucleotide strand and tRNA (transfer RNA) is made of a single polynucleotide strand folded into a clover shape.
- That transcription is the first stage of protein synthesis and involves the production of an mRNA copy of a gene from DNA.
- That during transcription, DNA strands separate and the enzyme RNA polymerase lines up free RNA nucleotides and joins them together to form an mRNA strand.
- That transcription in prokaryotes results in the direct production of mRNA from DNA. In eukaryotes, transcription produces pre-mRNA.
- That pre-mRNA contains introns. These non-coding introns are removed by splicing to form mRNA — this leaves only exons present in the mRNA.
- That translation is the second stage of protein synthesis in which amino acids are joined together by ribosomes to make a polypeptide strand (protein) based on the order of codons in mRNA.
- That tRNA molecules carry amino acids to the ribosomes during translation.
- That ATP is needed to provide the energy for the bond formation between the amino acid and the tRNA molecule to form, allowing the tRNA to carry the amino acid during translation.
- That the genetic code is universal (the same base pairs code for the same amino acids in all living things), non-overlapping (codons do not share triplets) and degenerate (there are more possible combinations of triplets than there are amino acids).
- How to relate the base sequence of nucleic acids to the amino acid sequence of the polypeptides that they code for.
- How to interpret experimental data relating to the role of nucleic acids (e.g. data from the investigation of transcription and/or translation).

Exam-style Questions

- 1 A species of bacteria has a gene that codes for the production of a blue-coloured antibiotic.
- 1.1 Describe the role of RNA polymerase in the transcription of a gene sequence.
(2 marks)
- 1.2 The mRNA for the gene coding for the antibiotic is the same length as its DNA. Explain how and why this might be different for a eukaryotic gene.
(3 marks)
- 2 Researchers have been studying the genetic code of a gene with the aim of developing a treatment for a particular genetic disease.
- 2.1 What is the genetic code?
(1 mark)
- 2.2 The genetic code is described as universal and degenerate. Explain what these terms mean.
(2 marks)
- The genetic disease is caused by the production of a specific enzyme. Part of the enzyme's amino acid sequence is shown below.
- Glycine — Histidine — Alanine
— Proline — Histidine
- Table 1** shows the DNA sequence for some amino acids.
- 2.3 Use **Table 1** to give the tRNA anticodons for the amino acid sequence shown above.
(2 marks)
- 2.4 Describe how the structure of tRNA differs from mRNA.
(3 marks)
- The researchers are exploring a possible treatment for the genetic disease that would involve disrupting the process of translation.
- 2.5 Name the organelle that mRNA attaches to for translation to take place.
(1 mark)
- 2.6 Give a detailed description of tRNA's role in translation.
(4 marks)

| Amino acid | DNA sequence |
|------------|--------------|
| Valine | CAG |
| Proline | GGA |
| Glutamine | GTT |
| Histidine | GTG |
| Glycine | CCT |
| Serine | TCG |
| Alanine | CGT |

1. Meiosis and Genetic Variation

Most cells in the body contain exactly the same genetic information. The one major exception to this rule is the gametes — the cells involved in sexual reproduction.

Diploid body cells

Normal body cells have the **diploid number ($2n$)** of chromosomes — meaning each cell contains two of each chromosome (a pair), one from the mum and one from the dad.

The chromosomes that make up each pair are the same size and have the same genes, although they could have different versions of those genes (called alleles). These pairs of matching chromosomes are called **homologous pairs** — see page 205. Humans have 23 homologous pairs and so 46 chromosomes in total. Therefore the diploid number for humans is 46.

Gametes and sexual reproduction

Gametes are the sperm cells in males and egg cells in females. Gametes have a **haploid (n) number** of chromosomes — they only contain one copy of each chromosome in a homologous pair. The haploid number for humans is 23.

In sexual reproduction two gametes join together at fertilisation to form a zygote, which divides and develops into a new organism.

Fertilisation

At fertilisation, a haploid sperm fuses with a haploid egg, making a cell with the normal diploid number of chromosomes (see Figure 1). Half these chromosomes are from the father (the sperm) and half are from the mother (the egg).

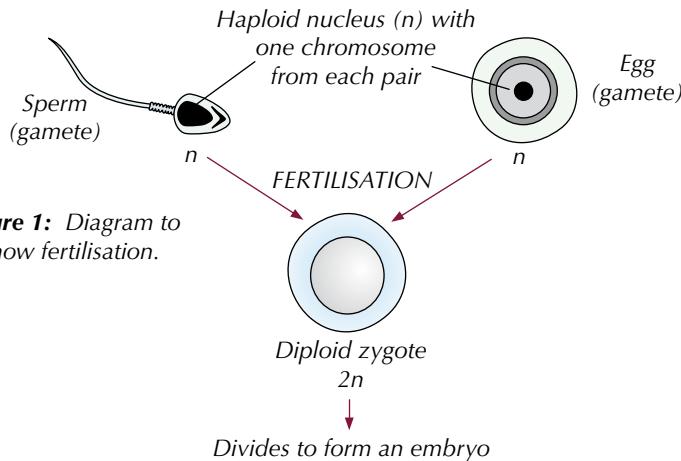


Figure 1: Diagram to show fertilisation.

During sexual reproduction, any sperm can fertilise any egg — fertilisation is random. Random fertilisation produces zygotes with different combinations of chromosomes to both parents. This mixing of genetic material in sexual reproduction increases genetic diversity within a species (there's more on genetic diversity on page 226).

Learning Objectives:

- Be able to explain how the random fertilisation of haploid gametes increases genetic variation within a species.
- Know that meiosis produces daughter cells that are genetically different from each other.
- Know that in meiosis, two nuclear divisions usually result in the formation of four haploid daughter cells from a single diploid parent cell.
- Know that genetically different daughter cells result from independent segregation in meiosis.
- Know that crossing over results in further genetic variation among daughter cells.
- Be able to complete diagrams showing the chromosome content of cells after the first and second meiotic divisions, when given the chromosome content of the parent cell.
- Be able to recognise where meiosis occurs when given information about an unfamiliar life cycle.
- Be able to explain the different outcomes of mitosis and meiosis.

Specification Reference 3.4.3

Tip: In humans, meiosis takes place in the ovaries and testes.

Tip: All the different chromosome-related terms can get a bit confusing — this diagram might help:

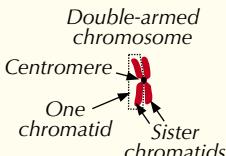


Figure 2: Condensed double-armed chromosomes.

Tip: Remember, in the first division, the homologous pairs separate. In the second division, the chromatids separate.



Figure 3: Chromatids separating during meiosis II.

Meiosis

Meiosis is a type of cell division. It takes place in the reproductive organs of multicellular, eukaryotic organisms. Cells that divide by meiosis are diploid to start with, but the cells that are formed from meiosis are haploid — the chromosome number halves. Meiosis in humans and other mammals produces gametes directly. In other organisms (e.g. some insects and plants) it produces haploid cells which later divide by mitosis to become gametes. Without meiosis, you'd get double the number of chromosomes when the gametes fused. Not good. The process of meiosis is summarised in Figure 4.

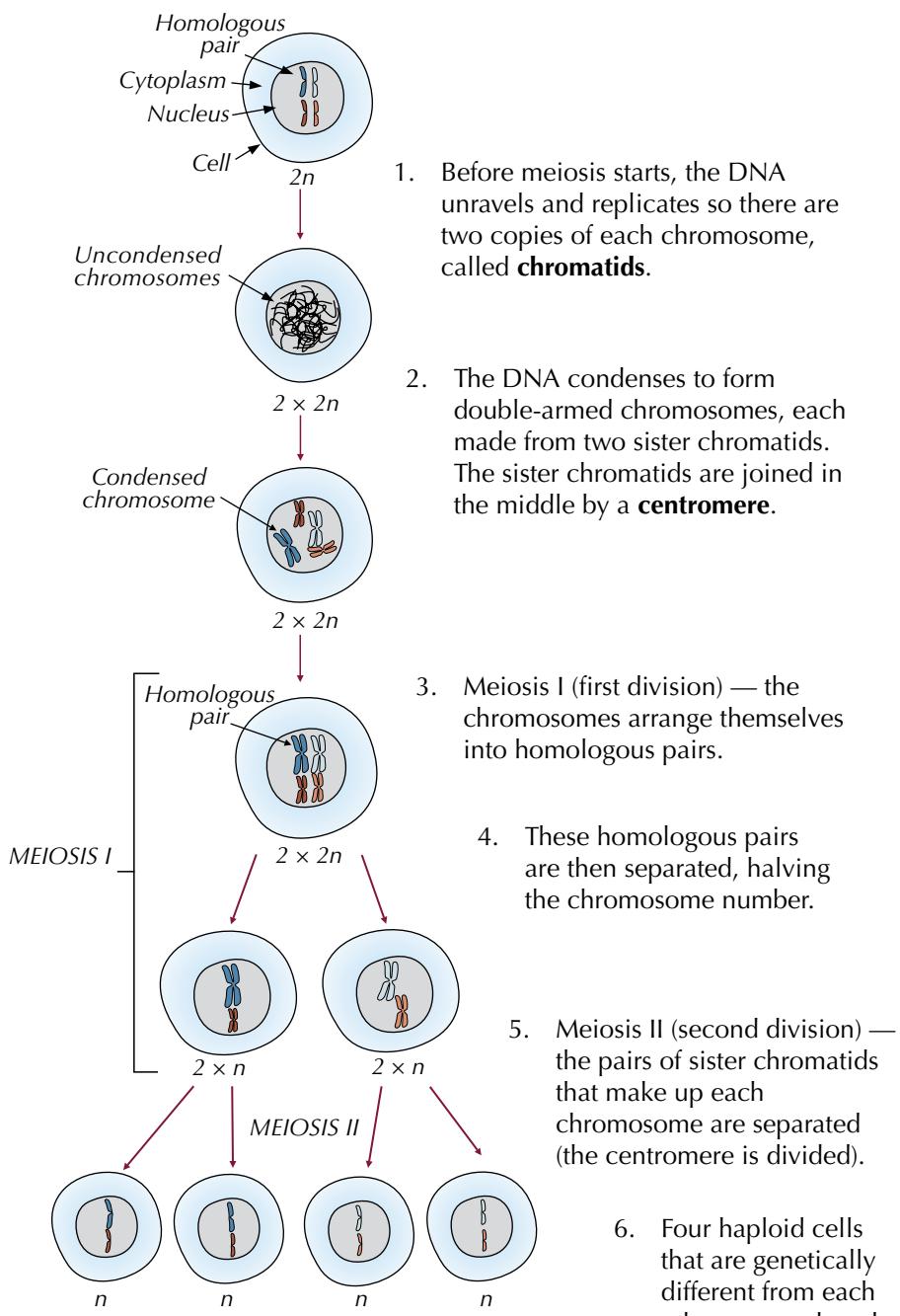


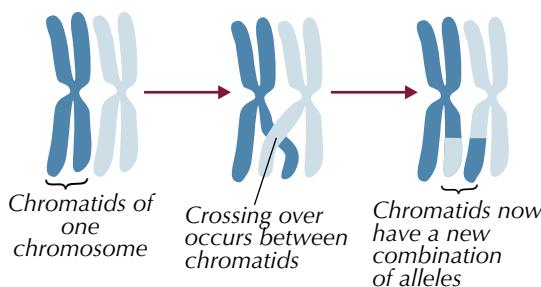
Figure 4: Diagram showing the different stages of meiosis in a diploid cell with four chromosomes.

Creating genetic variation in gametes

There are two main events during meiosis that lead to genetic variation:

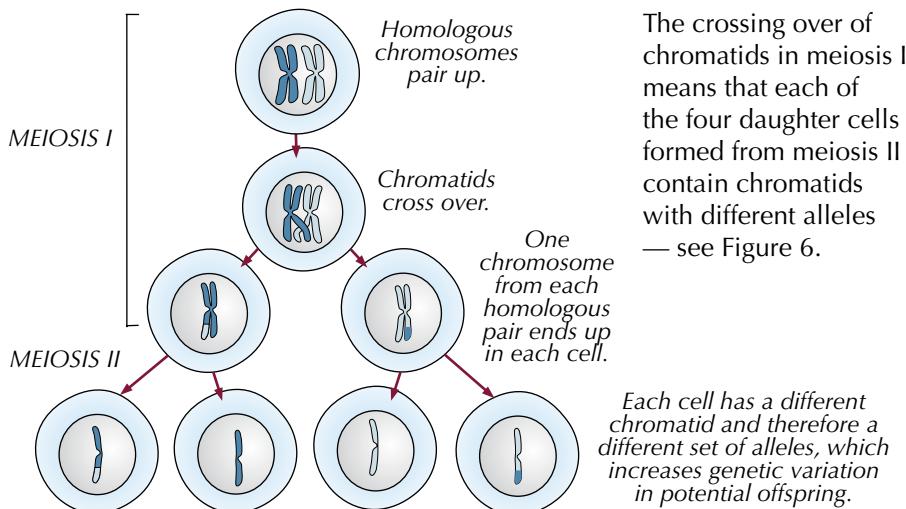
1. Crossing over of chromatids

During meiosis I, homologous chromosomes come together and pair up. The chromatids twist around each other and bits of chromatids swap over. The chromatids still contain the same genes but now have a different combination of alleles — see Figure 5.



Tip: Crossing over is also known as recombination.

Figure 5: Crossing over.



Each cell has a different chromatid and therefore a different set of alleles, which increases genetic variation in potential offspring.

Figure 6: Crossing over in meiosis.

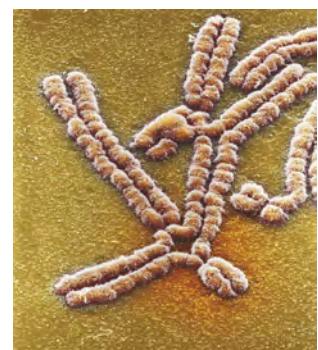
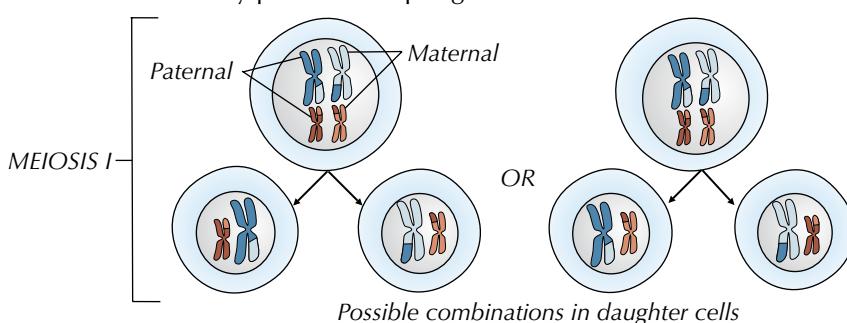


Figure 7: Electron micrograph showing crossing over occurring in cells.

2. Independent segregation of chromosomes

Each homologous pair of chromosomes in your cells is made up of one chromosome from your mum (maternal) and one chromosome from your dad (paternal). When the homologous pairs are separated in meiosis I, it's completely random which chromosome from each pair ends up in which daughter cell. So the four daughter cells produced by meiosis have completely different combinations of those maternal and paternal chromosomes (see Figure 8). This is called independent segregation (separation) of the chromosomes. This 'shuffling' of chromosomes leads to genetic variation in any potential offspring.



Tip: In any diploid species there are 2^n possible combinations of maternal and paternal chromosomes (where n is the number of homologous pairs). This means that in humans (which have 23 homologous pairs) there are 2^{23} or 8 388 608 possible combinations of chromosomes.

Figure 8: Independent segregation of chromosomes.

Life cycles

In the exams, you might need to spot when meiosis happens in an organism with a life cycle you haven't seen before, e.g. an insect or plant. Just remember that in any organism, meiosis is needed for sexual reproduction because it produces daughter cells (usually gametes) with half the number of chromosomes of the parent cell.

Tip: The malaria parasite is a single-celled eukaryotic organism called a protist. Prokaryotes don't divide by meiosis or reproduce sexually.

Tip: Compare the number of chromosomes in the parent and daughter cells — if they halve, the cell must have undergone meiosis.

Tip: Different species have different numbers of chromosomes, so '2n' and 'n' represent different numbers in different species. E.g. in mosquitoes $2n = 14$. In humans $2n = 46$.

Tip: Zygotes in humans divide by mitosis, not by meiosis. Don't let this put you off. The important thing here is what's happening to the chromosome number.

Tip: Meiosis doesn't produce gametes directly in the mosquito life cycle. It still produces haploid cells though.

Example

The stages in the life cycle of the malaria parasite are shown in Figure 9.

When and where does meiosis take place in the parasite's life cycle?

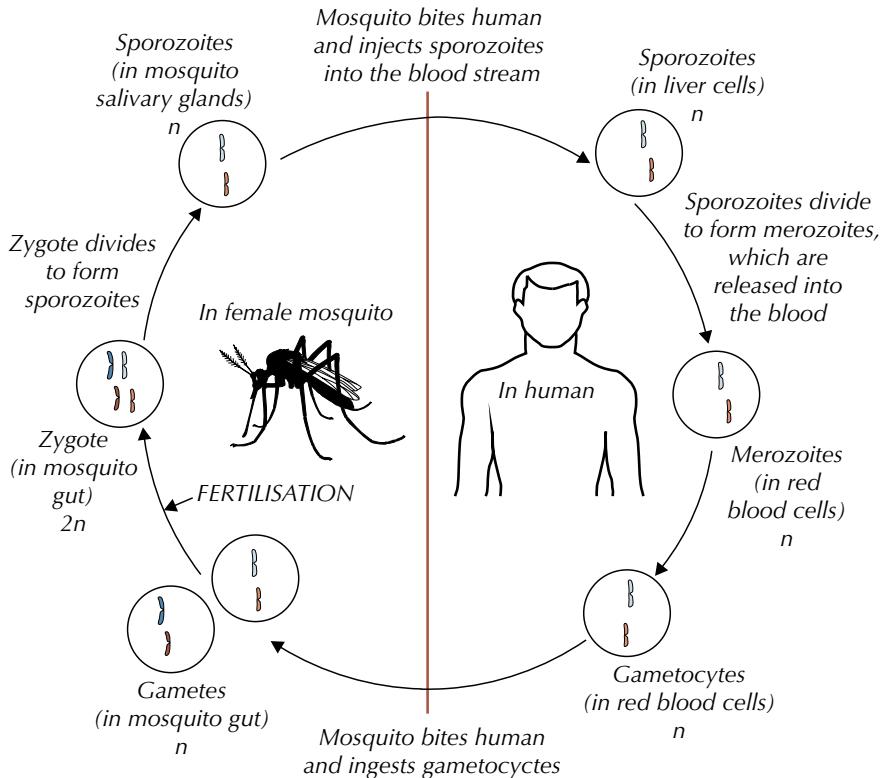


Figure 9: The life cycle of the malaria parasite.

- Only the zygote of the malarial parasite is diploid ($2n$). All the other stages of its life cycle are haploid (n).
- The chromosome number only halves when the diploid zygote divides in the mosquito's gut to form haploid sporozoites. In all the other divisions in the parasite's life cycle, the chromosome number stays the same — a haploid cell forms another haploid cell, e.g. haploid sporozoites divide (by mitosis) to form haploid merozoites.
- So only the zygote divides by meiosis and it only happens in the mosquito's gut.
- Fertilisation of the gametes (sexual reproduction) also takes place in the mosquito's gut.

You might also be told how many chromosomes are in a parent cell, then asked to complete diagrams showing how many chromosomes will be in the daughter cells after the first and second divisions of meiosis. Remember that the chromosome number is halved during the first division.

Outcomes of mitosis and meiosis

You may remember mitosis from pages 86-87. It's part of the cell cycle — the process multicellular organisms use to grow and divide. Mitosis and meiosis have different outcomes. These are shown in Figure 10.

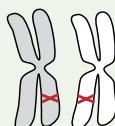
| | Outcomes: | | |
|---------|--|--|-------------------------------|
| Mitosis | Produces cells with the same number of chromosomes as the parent cell. | Daughter cells are genetically identical to each other and to the parent cell. | Produces two daughter cells. |
| Meiosis | Produces cells with half the number of chromosomes as the parent cell. | Daughter cells are genetically different from one another and the parent cell. | Produces four daughter cells. |

Figure 10: Table comparing outcomes in mitosis and meiosis.

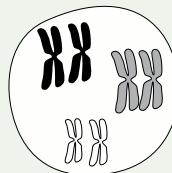
You need to be able to explain the different outcomes of mitosis and meiosis. They're different because mitosis only involves one division (which separates the sister chromatids) whereas meiosis has two divisions (which separate the homologous pairs and then the sister chromatids). There's no pairing or separating of homologous chromosomes in mitosis, and so no crossing over or independent segregation of chromosomes. This produces genetically identical daughter cells — unlike meiosis.

Practice Questions — Application

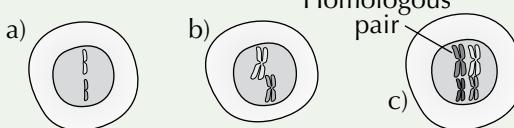
- Q1 The diagram below shows two homologous chromosomes. The red cross marks a point at which crossing over can occur. Draw the chromosomes as they would be if crossing over occurred at this point.



- Q2 The diagram below shows a cell that contains three pairs of homologous chromosomes. Draw a viable gamete that could be produced by this cell after meiosis.



- Q3 For each of the following cells state what stage the cell is at in meiosis. Choose from: before meiosis I, between meiosis I and II, or after meiosis II. Give a reason for each answer.



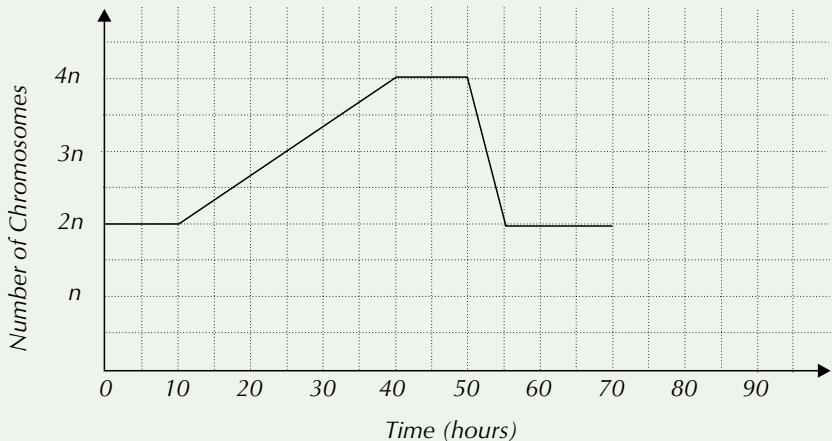
Exam Tip

Make sure you know what the chromosomes look like in each stage of meiosis.

Exam Tip

Examiners just love a good graph — so make sure you practise interpreting them.

Q4 The graph below shows the average DNA content of a group of cells that are undergoing meiosis:



- Describe what is happening:
 - between 10 hours and 40 hours.
 - between 40 hours and 50 hours.
 - between 50 and 55 hours.
- Sketch a line on the graph to show what is likely to happen to the DNA content of the cell between 70 and 95 hours.

Practice Questions — Fact Recall

- Q1 Are the following haploid or diploid:
a) normal body cells? b) gametes? c) zygotes?
- Q2 Outline what happens in: a) meiosis I. b) meiosis II.
- Q3 a) What are the two main events in meiosis that lead to genetic variation?
b) Describe how each of these processes works.
- Q4 Give three differences in the outcomes of mitosis and meiosis.

2. Mutations

Sometimes things don't quite go as they're supposed to in cell replication — gene mutations and mutations in the number of chromosomes can occur.

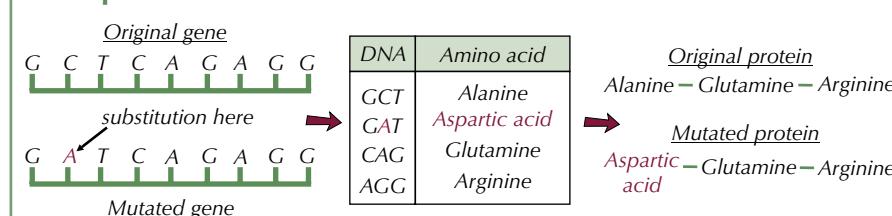
Gene mutations

Gene mutations involve a change in the DNA base sequence of chromosomes. The types of errors that can occur include:

- Substitution — one base is substituted with another,
e.g. ATGCCT becomes ATTCCT (G is swapped for T).
- Deletion — one base is deleted,
e.g. ATGCCT becomes ATCCT (G is removed).

The order of DNA bases in a gene determines the order of amino acids in a particular protein (see p. 204). If a mutation occurs in a gene, the sequence of amino acids it codes for (and the protein formed) could be altered:

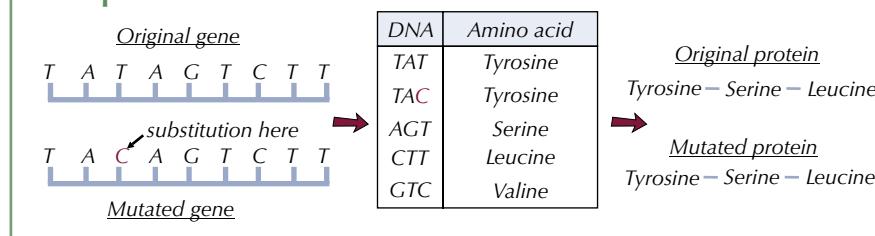
Example



Effects of mutations

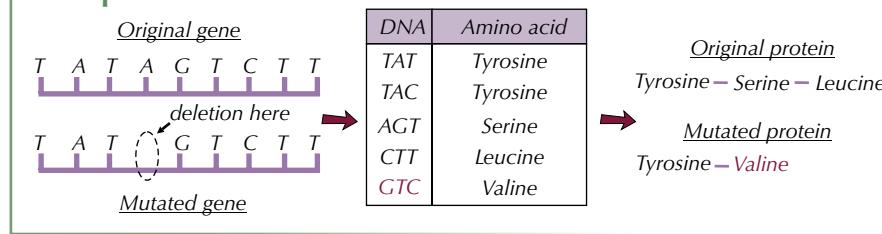
The degenerate nature of the genetic code (see page 211) means that some amino acids are coded for by more than one DNA triplet (e.g. tyrosine can be coded for by TAT or TAC in DNA). This means that not all substitution mutations will result in a change to the amino acid sequence of the protein — some substitutions will still code for the same amino acid.

Example



Substitution mutations won't always lead to changes in the amino acid sequence, but deletions will — the deletion of a base will change the number of bases present, which will cause a shift in all the base triplets after it.

Example



Learning Objectives:

- Know that gene mutations involve a change in the base sequence of chromosomes.
- Know that gene mutations can arise spontaneously during DNA replication and include base deletion and base substitution.
- Understand that due to the degenerate nature of the genetic code, not all base substitutions cause a change in the sequence of encoded amino acids.
- Know that mutagenic agents can increase the rate of gene mutation.
- Know that mutations in the number of chromosomes can arise spontaneously by chromomere non-disjunction during meiosis.

Specification Reference 3.4.3

Tip: Remember, three DNA bases (a triplet) code for one amino acid.

Tip: Errors can also be caused by insertion, duplication, addition and translocation of bases. You'll learn more about these in Year 2 of A-level Biology.

Mutagenic agents

Tip: X-rays and gamma rays are examples of ionising radiation.

Tip: Mutations are always random — only the rate of mutation is affected by mutagenic agents.

Mutations occur spontaneously, e.g. when DNA is misread during replication. But some things can cause an increase in the rate of mutations — these are called mutagenic agents. Ultraviolet radiation, ionising radiation, some chemicals and some viruses are examples of mutagenic agents. Mutagenic agents increase the probability of a mutation occurring.

Example — Maths Skills

The **chance** of something happening is the possibility that it will occur. **Probability** is a measure of how likely events are to happen. In maths, the probability of any event happening has to be between 0 (the event is impossible) and 1 (the event is certain).

There is always a chance of gene mutations occurring. However, under normal circumstances, the probability of a mutation occurring at any particular point is very low. This probability is increased by exposure to mutagenic agents, such as ultraviolet radiation. You can't predict where exactly in the DNA the mutation will occur, but the likelihood of a mutation occurring somewhere is increased when an organism is exposed to UV radiation.

Chromosome mutations

Tip: Non-disjunction leading to Down's syndrome can occur in meiosis I, as shown in Figure 2, or in meiosis II.

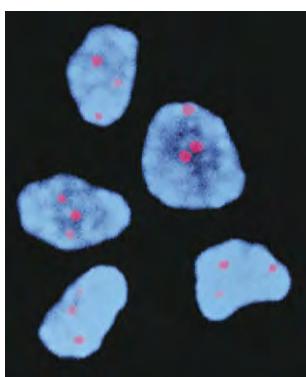


Figure 1: Three copies of chromosome 21 (pink) are seen in each of the cell nuclei (blue) of a fetus.

In humans, when meiosis works properly, all four daughter cells will end up with 23 whole chromosomes — one from each homologous pair (1 to 23). But sometimes meiosis goes wrong and the cells produced contain variations in the numbers of whole chromosomes or parts of chromosomes. E.g. two cells might have 23 whole chromosomes, one each of 1 to 23, but the other two might get a bit muddled up, one having two chromosome 6's and the other no chromosome 6. This is called chromosome mutation and is caused by errors during meiosis. Chromosome mutations lead to inherited conditions because the errors are present in the gametes (the hereditary cells). One type of chromosome mutation is called **chromosome non-disjunction** — it's a failure of the chromosomes to separate properly.

Example

Down's syndrome is caused by a person having an extra copy of chromosome 21 (or sometimes an extra copy of part of chromosome 21). Non-disjunction means that chromosome 21 fails to separate properly during meiosis, so one cell gets an extra copy of 21 and another gets none. When the gamete with the extra copy fuses to another gamete at fertilisation, the resulting zygote will have three copies of chromosome 21 (see Figure 2).

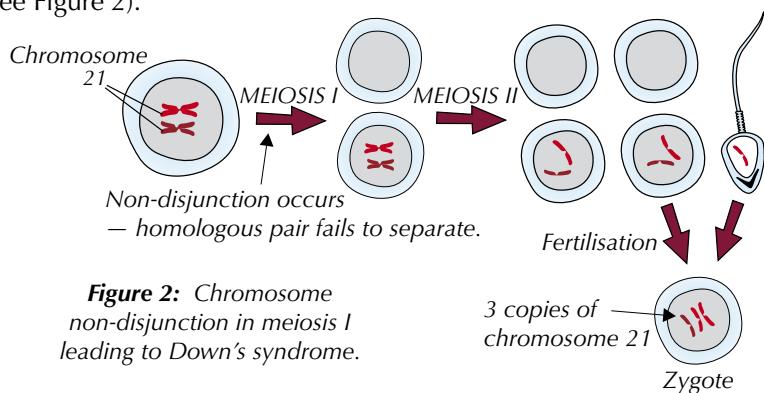


Figure 2: Chromosome non-disjunction in meiosis I leading to Down's syndrome.

Practice Questions — Application

- Q1 The diagram on the right shows part of a gene sequence that has been mutated.
- Describe the mutation that has occurred.
 - Using the table on the right, give the amino acid sequences of the original and mutated genes.
 - The diagram below shows the mutation of a different gene sequence. Explain why the mutation results in the same amino acid sequence.

Original gene *Mutated gene*
TATAGTGAG TACAGTGAG

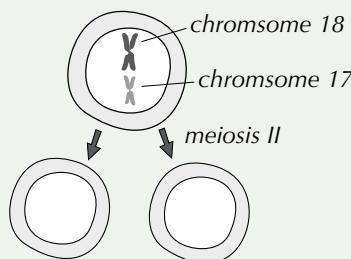
Original gene
CTTGAGTAC

Mutated gene
CTTAGTAC

| DNA | Amino acid |
|-----|---------------|
| TAT | Tyrosine |
| TAC | Tyrosine |
| GAA | Glutamic acid |
| GAG | Glutamic acid |
| AGT | Serine |
| CTT | Leucine |
| TTT | Phenylalanine |

Tip: You'll need to use the table in Q1 to help you answer part c) too.

- Q2 Edwards' syndrome is caused by a person having an extra copy of chromosome 18.
- Name the event that occurs during meiosis, which causes a person to have an extra copy of a chromosome.
 - Explain how this event could lead to a person having an extra copy of chromosome 18.
 - Complete the diagram below showing what would happen if the event took place during meiosis II and explain what briefly what it shows.



Practice Questions — Fact Recall

- What are gene mutations?
- Which type of gene mutations will always lead to a change in the amino acid sequence?
- a) What effect do mutagenic agents have on mutations?
b) Give an example of a mutagenic agent.

Learning Objectives:

- Know that genetic diversity is the number of different alleles of genes in a population.
- Understand that genetic diversity is a factor enabling natural selection to occur.

Specification Reference 3.4.4

Tip: All members of a species have the same genes. Diversity only occurs in the form of different alleles of those genes.

Tip: The gene pool is the complete range of alleles in a population.



Figure 2: Northern elephant seals in California.

3. Genetic Diversity

Meiosis generates genetic diversity, but it isn't the only thing that affects it.

What is genetic diversity?

Remember, there can be different versions of a single gene — these are called alleles (see page 204). Alleles code for different versions of characteristics, e.g. blonde hair or brown hair. **Genetic diversity** is the number of different alleles of genes in a species or population. A large number of different alleles in a population means a large variety of different characteristics (e.g. blonde, brown, red or black hair) and a high genetic diversity.

Genetic diversity is important — if a population has low genetic diversity, it might not be able to adapt to a change in the environment and the whole population could be wiped out by a single event (e.g. a disease). Genetic diversity within a population is increased by:

- Mutations in the DNA forming new alleles. Some of these can be advantageous, whilst others lead to problems (see page 89).
- Different alleles being introduced into a population when individuals from another population migrate into it and reproduce. This is known as gene flow.

Genetic diversity is what allows natural selection to occur (see page 228) because some characteristics are more advantageous than others.

Genetic bottlenecks

A genetic bottleneck is an event that causes a big reduction in a population, e.g. when a large number of organisms within a population die before reproducing. This reduces the number of different alleles in the **gene pool** and so reduces genetic diversity. The survivors reproduce and a larger population is created from a few individuals — see Figure 1.

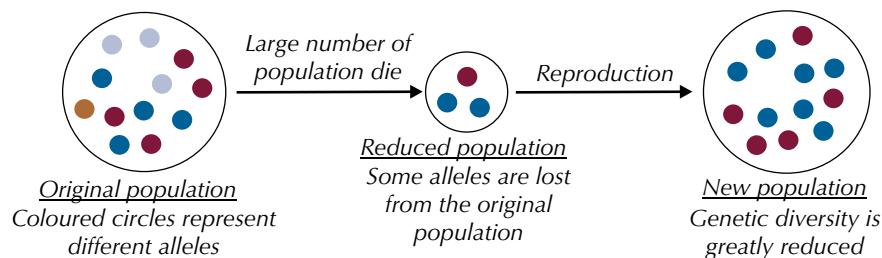


Figure 1: Diagram illustrating the effect of genetic bottlenecks.

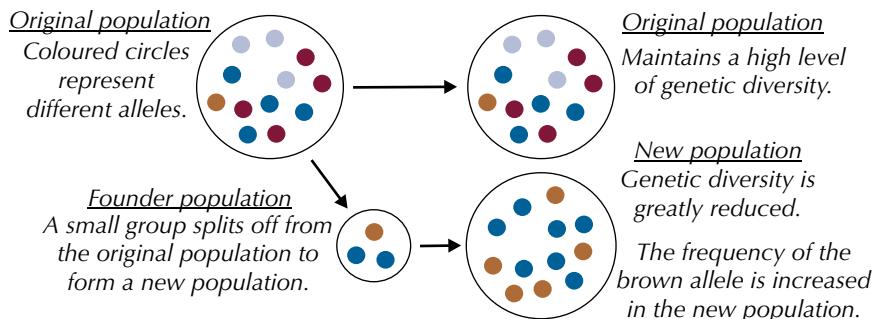
Example

Northern elephant seals were hunted by humans in the late 1800s. Their original population was reduced to around 50 seals who have since produced a population of around 170 000. This new population has very little genetic diversity compared to the southern elephant seals, which have not suffered such a reduction in numbers.

The founder effect

The founder effect describes what happens when just a few organisms from a population start a new colony and there are only a small number of different alleles in the initial gene pool (see Figure 3 on the next page).

The frequency of each allele in the new colony might be very different to the frequency of those alleles in the original population — for example, an allele that was rare in the original population might be more common in the new colony. This may lead to a higher incidence of genetic disease.



Tip: The brown allele in Figure 3 could represent a genetic disorder. It's easy to see why the founder effect can lead to an unusually high incidence of a certain genetic disorder, if the allele for it is present in the founding population.

Figure 3: Diagram illustrating the founder effect.

The founder effect can occur as a result of migration leading to geographical separation or if a new colony is separated from the original population for another reason, such as religion.

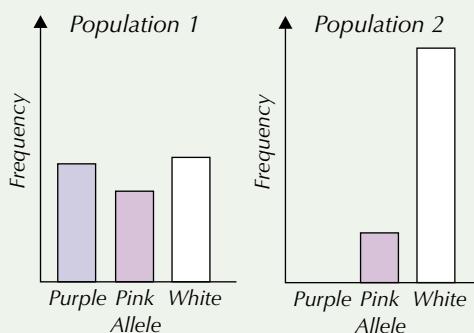
Example

The Amish population of North America are all descended from a small number of Swiss who migrated there. The population shows little genetic diversity. They have remained isolated from the surrounding population due to their religious beliefs, so few new alleles have been introduced. The population has an unusually high incidence of certain genetic disorders.

Practice Question — Application

Q1 Flowers of a plant species can be purple, pink or white. Each colour is coded for by a different allele. The graphs below show the frequencies of these alleles in two populations of the plant species.

- Based on this information alone, which population is more genetically diverse? Explain your answer.
- Explain how a genetic bottleneck could have led to the allele frequencies shown in Population 2.



Practice Questions — Fact Recall

- What is genetic diversity?
- Give two ways in which genetic diversity within a population can be increased.

Learning Objectives:

- Understand the principles of natural selection in the evolution of populations.
- Know that random mutation can result in new alleles of a gene.
- Know that many mutations are harmful but, in certain environments, the new allele might benefit its possessor, leading to increased reproductive success.
- Understand that the advantageous allele is inherited by members of the next generation and that over many generations, the new allele increases in frequency in the population.
- Be able to use unfamiliar information to explain how selection produces changes within a population of a species.
- Know that natural selection results in species that are better adapted to their environment and that adaptations may be anatomical, physiological or behavioural.
- Understand that adaption and selection are major factors in evolution and contribute to the diversity of living organisms.

Specification Reference 3.4.4

4. Natural Selection

Variation gives some organisms an advantage over others. Natural selection increases the proportion of the advantageous alleles within a population.

The process of natural selection

Randomly-occurring mutations sometimes result in a new allele being formed. This can be harmful, which usually means that the mutated allele quickly dies out. However, some mutations can produce alleles that are beneficial to an organism (e.g. a protein is produced that works better than the original), helping the organism to survive in certain environments.

When the allele codes for a characteristic that increases the chances of an organism surviving, its frequency within the population can increase. This process is known as **natural selection**. Here's how it works:

1. Not all individuals are as likely to reproduce as each other. In other words, there's **differential reproductive success** in a population — individuals that have an allele that increases their chance of survival are more likely to survive, reproduce and pass on their genes (including the beneficial allele), than individuals with less advantageous alleles.
2. This means that a greater proportion of the next generation inherits the beneficial allele.
3. They, in turn, are more likely to survive, reproduce and pass on their genes.
4. So the frequency of the beneficial allele in the population increases from generation to generation.
5. Over generations this leads to **evolution** as the advantageous alleles become more common in the population.

Evolution is the gradual change in species over time. It has led to the huge diversity of living organisms on Earth. Adaptation and selection are both key factors in evolution.

Example

In 1810 a herd of caribou were taken from the Arctic to an area with a warmer climate.

In 1810 the average fur length was 3.5 cm.

In 1960 it was 2.1 cm. This change can be explained by natural selection:

- There is variation in fur length in the population of caribou — mutations in the fur length gene mean some caribou have an allele for shorter fur and some have an allele for longer fur.
- Caribou with shorter fur will be better adapted to the warmer climate as they'll be less likely to overheat. These caribou will be more likely to survive, reproduce and pass on their genes (including the allele for shorter fur) than caribou with the allele for longer fur.
- This means that a greater proportion of the next generation will inherit the allele for shorter fur, so the frequency of this allele will increase from generation to generation.
- Over many generations this leads to evolution, as the advantageous allele for short fur becomes more common in the population.



Figure 1: Grazing caribou.

Types of adaptations

Natural selection leads to organisms becoming better adapted to their environment. **Adaptations** are features that help organisms to survive in their environment. They can be behavioural, physiological or anatomical.

Tip: Organisms that are well adapted to their environment have a **selective advantage** over less-well adapted organisms.

1. Behavioural adaptations

These are ways an organism acts that increase its chance of survival.

Examples

- Possums sometimes ‘play dead’ — if they’re being threatened by a predator they play dead to escape attack. This increases their chance of survival.
- Scorpions dance before mating — this makes sure they attract a mate of the same species, increasing the likelihood of successful mating.



Figure 2: When American possums feel threatened, they ‘play dead’ to escape attack.

2. Physiological adaptations

These are processes inside an organism’s body that increase its chance of survival.

Examples

- Brown bears hibernate — they lower their rate of metabolism (all the chemical reactions taking place in their body) over winter. This conserves energy, so they don’t need to look for food in the months when it’s scarce — increasing their chance of survival.
- Some bacteria produce antibiotics — these kill other species of bacteria in the area. This means there’s less competition, so they’re more likely to survive.



Figure 3: An otter’s streamlined body helps it to move easily through water.

3. Anatomical (structural) adaptations

These are structural features of an organism’s body that increase its chance of survival.

Examples

- Otters have a streamlined shape — making it easier to glide through the water. This makes it easier for them to catch prey and escape predators, increasing their chance of survival.
- Whales have a thick layer of blubber (fat) — this helps to keep them warm in the cold sea. This increases their chance of survival in places where their food is found.



Figure 4: A black rat snake climbing up a tree.

Practice Questions — Application

Q1 There are many different species of rat snake, all found in different habitats and with slightly different colourings. The black rat snake lives in wooded habitats and has a dark, brown-black colouring (see Figure 4).

Describe how natural selection could explain the evolution of a rat snake with black colouring in a wooded habitat.

Exam Tip

No matter what example you get given in the exam, the important thing to remember is that all adaptations help organisms to survive, reproduce and pass on their alleles.

Exam Tip

In the exam, you need to write that the frequency of the beneficial allele increases over many generations.

- Q2 DDT is a chemical insecticide that was first used to kill malaria-carrying mosquitos around the time of WWII. In the 1950s, DDT-resistant mosquitos began to appear in areas of widespread DDT use.
Describe how DDT-resistance became widespread in some mosquito populations.
- Q3 Killer whales are commonly found in the cold seas around the Arctic and Antarctic. Like all whales, they are mammals. They can't breathe underwater, so have to hold their breath while they dive. They live and hunt in groups called pods, eating a varied diet of fish, seals, sea lions and other whales. Killer whales dive to catch their prey and can reduce their heart rate by up to half whilst diving. A thick layer of blubber (fat) under the whales' skin gives them a smooth, rounded shape.
- Using the information given above:
- Name one behavioural, one physiological and one anatomical adaptation of the killer whale to its environment.
 - For each adaptation, explain how it helps the killer whale to survive.

Practice Questions — Fact Recall

- Describe the role of random mutations in natural selection.
- Describe how natural selection increases the frequency of advantageous alleles in a population.
- What is an adaptation?
- Explain what is meant by the term 'physiological adaptations'.

5. The Effects of Selection

Natural selection affects different populations in different ways, leading to different allele frequencies...

Types of selection

Natural selection alters allele frequency in a population — see page 228. Directional selection and stabilising selection are types of natural selection that affect allele frequency in different ways.

1. Directional selection

Directional selection is where individuals with alleles for characteristics of an extreme type are more likely to survive and reproduce. This could be in response to an environmental change.

Example — Bacteria evolving antibiotic resistance

Some individuals in a bacterial population have alleles that give them resistance to an antibiotic. The population is exposed to the antibiotic, killing bacteria without the resistance allele.

The resistant bacteria survive and reproduce without competition, passing on the allele that gives antibiotic resistance to their offspring. After some time, most organisms in the population will carry the antibiotic resistance allele — see Figure 1.

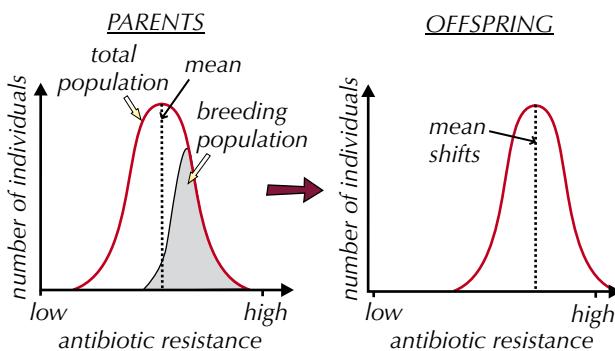


Figure 1: Graphs to show directional selection in bacteria.

2. Stabilising selection

Stabilising selection is where individuals with alleles for characteristics towards the middle of the range are more likely to survive and reproduce. It occurs when the environment isn't changing, and it reduces the range of possible characteristics.

Example — Human birth weight

Humans have a range of birth weights. Very small babies are less likely to survive — they have a high surface area to volume ratio, which means they find it hard to maintain their body temperature. This puts pressure on their respiratory and cardiac systems, which can be fatal.

Very large babies are less likely to survive too. Giving birth to large babies can be difficult because their large size makes it harder for them to fit through the mother's pelvis. This can lead to complications for both mother and child.

Conditions are most favourable for medium-sized babies — so the weight of human babies tends to shift towards the middle of the range — see Figure 2 on the next page.

Learning Objectives:

- Understand the process of directional selection, exemplified by antibiotic resistance in bacteria.
- Understand the process of stabilising selection, exemplified by human birth weights.
- Be able to interpret data relating to the effects of selection in producing change within populations.

Specification Reference 3.4.4

Tip: Bacteria can also evolve resistance to other chemicals that are designed to kill them, e.g. antiseptics.

Exam Tip

Make sure you learn both of the examples on this page for the exams.

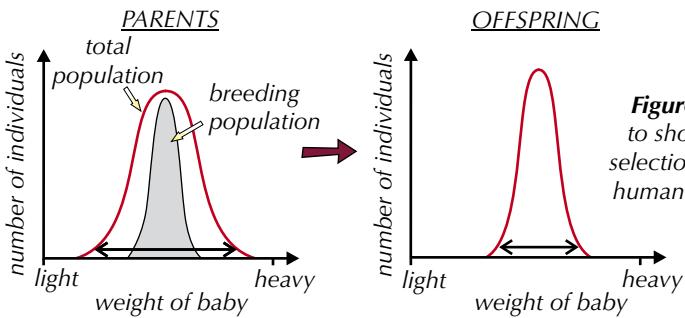


Figure 2: Graphs to show stabilising selection as shown by human birth weights.

Interpreting data on the effects of selection

You might be asked to interpret data on selection in the exam.

Example

Exam Tip

If the variation stays roughly the same but the mean changes, it must be an example of directional selection. If the mean stays the same but the variation decreases, you are looking at an example of stabilising selection.

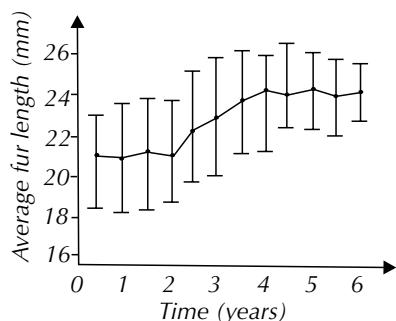
A population of rabbits has varying fur length. Longer fur helps to keep the rabbits warmer. The graph below shows how the average fur length of the rabbits changed over a period of six years, which had particularly cold winters. The bars span the difference between the shortest and longest fur lengths recorded.

Describe what the data shows:

Over the first two years the average fur length is about 21 mm. However, the average length gradually increases from 21 mm to 24 mm. This shows directional selection.

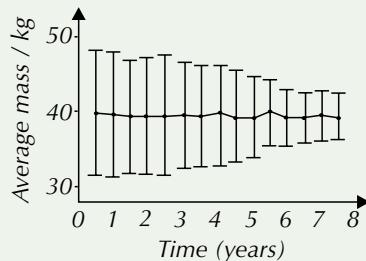
Suggest a possible cause:

The rabbits with the longer fur are more likely to survive the cold winters than the short-furred rabbits. This makes them more likely to reproduce and, when they do, they pass on the allele for longer fur to the next generation. Over time, the allele for longer fur becomes more common in the population and the average fur length of the rabbits increases.



Practice Question — Application

Q1 The graph on the right shows how the average mass in an isolated population of wolves changed over a period of 8 years. The vertical bars span the difference between the lightest and heaviest wolves. The wolves lived in a snowy habitat.



- Is this an example of directional or stabilising selection? Give a reason for your answer.
- Suggest why these changes occurred in the population of wolves.

Tip: Try to think about why smaller or larger wolves might be less likely to survive.

Practice Questions — Fact Recall

- What is directional selection?
- Bacteria evolving antibiotic resistance is an example of directional selection. Explain why.

6. Investigating Selection

You can carry out practical investigations into the effects of antimicrobial substances (substances that kill microorganisms, e.g. antibiotics, antiseptics or disinfectants) on microbes. These investigations should show you whether the microbes have evolved resistance to these substances or not.

Testing the effects of antibiotics

Antibiotics are medicines that are designed to kill bacteria.

This makes them a type of **antimicrobial substance**. You can investigate the effects of different antibiotics on bacterial growth using the following method.

The whole investigation must be carried out using **aseptic techniques**.

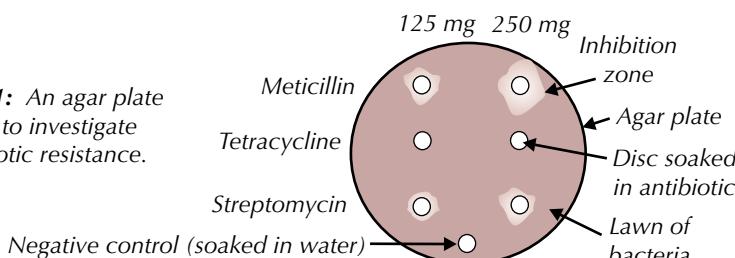
These are explained on the next page. Read them through before you begin.

1. The bacteria you will use are likely to have been grown in a liquid broth (a mixture of distilled water, bacterial culture and nutrients).
2. Use a sterile pipette to transfer the bacteria from the broth to an agar plate (a Petri dish containing agar jelly). Spread the bacteria over the plate using a sterile plastic spreader.
3. Use sterile forceps to place paper discs soaked with different antibiotics spaced apart on the plate. Various concentrations of antibiotics should be used. You also need to add a negative control disc soaked only in sterile water.
4. Tape a lid onto the Petri dish (without completely sealing it), invert, and incubate the plate at about 25°C for 48 hours. This allows the bacteria to grow, forming a 'lawn'. Anywhere the bacteria can't grow can be seen as a clear patch in the lawn of bacteria. This is called an **inhibition zone**.
5. The size of an inhibition zone tells you how well an antibiotic works. The larger the zone, the more the bacteria were inhibited from growing.

Example

Figure 1 shows an agar plate after it has been incubated with paper discs soaked in the antibiotics meticillin, tetracycline and streptomycin.

Figure 1: An agar plate used to investigate antibiotic resistance.



- The tetracycline discs have no inhibition zones, so the bacteria are resistant to tetracycline up to 250 mg.
- The streptomycin discs have small inhibition zones, with the zone at 250 mg slightly larger than the one at 125 mg. So streptomycin inhibits the growth of some of the bacteria.
- The meticillin discs have the largest inhibition zones, so meticillin inhibits the growth of most of the bacteria.
- The negative control has no inhibition zone, which shows that the other results must be due to the presence of the antibiotics, not the paper disc.

Learning Objective:

- Be able to use aseptic techniques to investigate the effect of antimicrobial substances on microbial growth (Required Practical 6).

Specification Reference 3.4.4

Tip: Make sure you carry out a full risk assessment before you carry out this practical. It's also really important that you understand how to use aseptic techniques properly before you start.

Tip: A negative control is not expected to have any effect on the experiment — see page 2 for more.

Tip: Don't completely seal the Petri dish with tape before incubation — it will prevent oxygen from entering the dish, which may encourage the growth of anaerobic disease-causing bacteria. Don't open the dish after incubation.



Figure 2: A bacterial culture plate with clear inhibition zones where an antibiotic has stopped the bacteria from growing.

Tip: Antiseptics and disinfectants are both non-selective chemicals that prevent the growth of microorganisms.

Antiseptics are used on living tissue, whereas disinfectants are used on non-living objects, e.g. kitchen surfaces.

Tip: Your teacher may also recommend other aseptic techniques, which you will need to follow. You should also take steps to protect yourself, such as wearing a lab coat and washing your hands thoroughly before and after handling any cultures.

A similar method can be used to test the effects of antiseptics or disinfectants on microbial growth — just replace the paper discs soaked in antibiotics with discs soaked in antiseptics or disinfectants.

Aseptic techniques

REQUIRED
PRACTICAL **6**

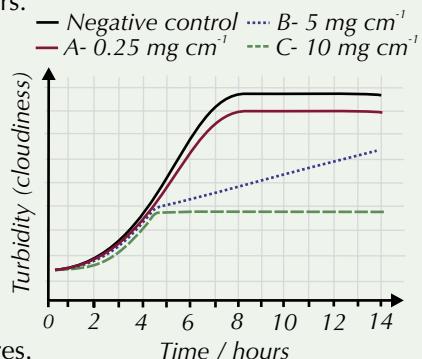
Aseptic techniques are used to prevent contamination of cultures by unwanted microorganisms. This is important because contamination can affect the growth of the microorganism that you're working with. It's also important to avoid contamination with disease-causing microbes that could make you ill. When carrying out the investigation on the previous page or any other investigation involving microorganisms, you need to use the following aseptic techniques:

- Regularly disinfect work surfaces to minimise contamination. Don't put any utensils on the work surface. Contaminated utensils should be placed in a beaker of disinfectant.
- Use sterile equipment and discard safely after use. E.g. glassware can be sterilised before and after use in an autoclave (which steams equipment at high pressure). Pre-sterilised plastic instruments are used once, then safely discarded.
- Work near a Bunsen flame. Hot air rises, so any microbes in the air should be drawn away from your plate.
- Minimise the time spent with the lid off the agar plate, to reduce the chance of airborne microorganisms contaminating the culture.
- Briefly flame the neck of the glass container of broth just after it's opened and just before it's closed — this causes air to move out of the container, preventing unwanted organisms from falling in.

Practice Question — Application

Q1 Turbidity is a measure of the cloudiness of a liquid. The more bacteria in a liquid, the cloudier it will be. A scientist grew some bacteria in a liquid broth. She then measured the turbidity of samples of the bacteria over time to see how an antibiotic affected growth. The results are shown below. The antibiotic (diluted in sterile water) was added at 4 hours.

- Suggest what the negative control might be and explain why it's used.
- Describe two aseptic techniques that the scientist would need to carry out in the preparation of the broth.
- Explain the importance of aseptic techniques when working with microbial cultures.
- Describe and explain what has happened to Sample C.



Practice Questions — Fact Recall

Q1 Give three examples of antimicrobial substances.

Q2 What substances would be present in a bacterial broth?

- Q3** a) What is an inhibition zone?
b) What does an inhibition zone tell you?

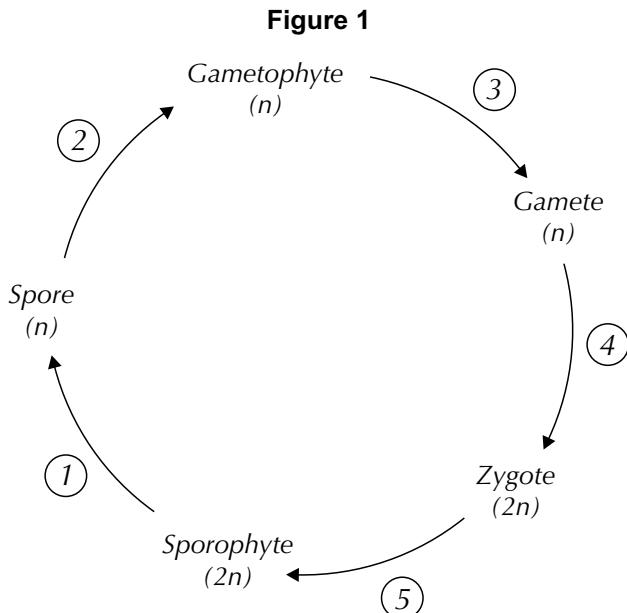
Section Summary

Make sure you know:

- That haploid gametes fuse at fertilisation to produce a diploid cell called a zygote.
- How the random fertilisation of gametes during sexual reproduction increases genetic diversity.
- That the process of meiosis involves two divisions — meiosis I (in which the homologous pairs separate) and meiosis II (in which the sister chromatids separate).
- That meiosis results in the formation of four haploid daughter cells from a single diploid parent cell and that the daughter cells are all genetically different from each other.
- How crossing over between homologous chromosomes in meiosis I leads to genetic variation among daughter cells.
- How independent segregation of homologous chromosomes in meiosis I results in the formation of genetically different daughter cells.
- How to recognise where meiosis occurs when given information about an unfamiliar life cycle.
- How to complete diagrams showing the chromosomal content of cells after meiosis I and meiosis II, when given the chromosome content of the parent cell.
- Why mitosis and meiosis have different outcomes.
- That gene mutations involve a change in the DNA base sequence of chromosomes.
- That gene mutations may involve base deletion or base substitution and that these can affect the sequence of amino acids coded for and therefore the protein that is produced.
- Why the degenerate nature of the genetic code means that not all substitutions result in a change in amino acid sequence in the protein, though deletions will.
- That gene mutations arise spontaneously during DNA replication.
- That the rate of mutation can be increased by mutagenic agents, such as ultraviolet radiation.
- That chromosome non-disjunction during meiosis can cause mutations in the number of chromosomes in a cell.
- That genetic diversity is the number of different alleles of genes in a species or population.
- That alleles introduced from other populations or by mutations can increase genetic diversity and that genetic bottlenecks and the founder effect can reduce genetic diversity.
- That random mutations can result in new alleles of a gene, which could be harmful or beneficial.
- That in the process of natural selection, individuals possessing an advantageous allele are more likely to survive, reproduce and pass on their genes to the next generation and that this process leads to an increase in frequency of the advantageous allele in a population over many generations.
- Why adaptation and selection are major factors in evolution and contribute to the diversity of living organisms.
- That natural selection results in species that are better adapted to their environment and that these adaptations may be anatomical, physiological or behavioural.
- How directional selection in bacteria results in a shift in the mean towards antibiotic resistance.
- How stabilising selection causes human birth weights to shift towards the middle of the range.
- How to interpret data relating to the effect of selection in producing change within populations.
- How to use aseptic techniques to investigate the effect of antimicrobial substances on microbial growth (Required Practical 6) — e.g. testing the effects of antibiotics on bacterial growth.

Exam-style Questions

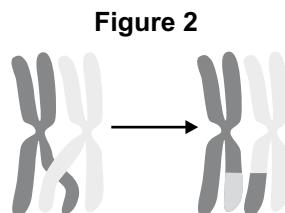
- 1 **Figure 1** shows the life cycle of a moss species.



- 1.1 At which point(s) in **Figure 1** is meiosis occurring?
Give a reason for your answer. (2 marks)
- 1.2 What is happening at point 4 of **Figure 1**? Give a reason for your answer. (2 marks)
- Genetic diversity in one population of this moss species is very low.
- 1.3 Suggest **one** reason why genetic diversity in this population may be low. (1 mark)
- 1.4 Give **two** ways that genetic diversity could be increased in a population of this moss species. (2 marks)

- 2 **Figure 2** shows two chromosomes during meiosis I.

- 2.1 Name the event taking place in **Figure 2**. (1 mark)
- 2.2 The event shown in **Figure 2** increases genetic variation in potential offspring. Name another event that takes place during meiosis that has the same effect and explain how this event increases genetic variation. (3 marks)

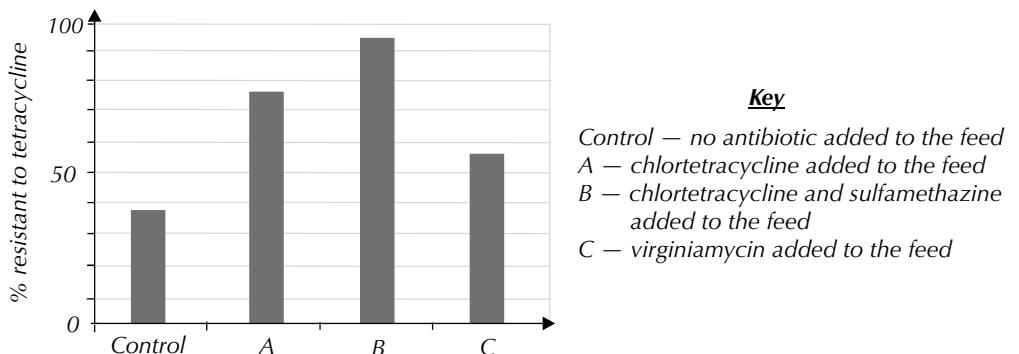


- 3 Mitosis and meiosis are both types of cell division.
Explain the different outcomes of mitosis and meiosis. (6 marks)
- 4 Species of *Streptomyces* bacteria are naturally found in soil, along with many other types of bacteria. They produce a wide range of clinically useful antibiotics including tetracycline-based antibiotics.
- 4.1 Use your knowledge of evolution by natural selection to explain how tetracycline-producing *Streptomyces* species may have become common in the population. (4 marks)
- 4.2 Species of *Streptomyces* also possess tet-resistance genes that protect them from the effects of tetracycline. For example, the *tetA* genes are responsible for pumping tetracycline out of cells, thereby protecting cells from its harmful effects.
- State whether tet-resistance is an example of directional or stabilising selection. Give a reason for your answer. (1 mark)

Tet-resistance is now found in a number of other species of bacteria. Scientists investigated the link between the use of antibiotics in cattle feed and the tetracycline resistance of *E. coli* samples isolated from the cattle. The samples were grown on agar plates and then tested for resistance to tetracycline.

The results are shown in **Figure 3**.

Figure 3



- 4.3 Suggest **two** factors the scientists should have considered when selecting cattle for the experiment. (2 marks)
- 4.4 Explain why the bacteria in the control sample were taken from cattle that had no antibiotics added to their feed. (1 mark)
- 4.5 What conclusions can be drawn from the results of the study? (3 marks)

Learning Objectives:

- Know that a phylogenetic classification system attempts to arrange species into groups based on their evolutionary origins and relationships. It uses a hierarchy in which smaller groups are placed within larger groups, with no overlap between groups. Each group is called a taxon (plural taxa).
 - Know that one hierarchy comprises the taxa: domain, kingdom, phylum, class, order, family, genus and species.
 - Know that two organisms belong to the same species if they are able to produce fertile offspring.
 - Know that each species is universally identified by a binomial consisting of the name of its genus and species, e.g. *Homo sapiens*.
- Specification Reference 3.4.5**



Figure 2: Chimps and gorillas are closely related.

1. Classification of Organisms

Scientists group related organisms together to make them easier to study. This is classification.

Phylogeny

Phylogeny is the study of the evolutionary history of groups of organisms. Phylogeny tells us who's related to whom and how closely related they are.

All organisms have evolved from shared common ancestors (relatives). This can be shown on a **phylogenetic tree** like the one in Figure 1.

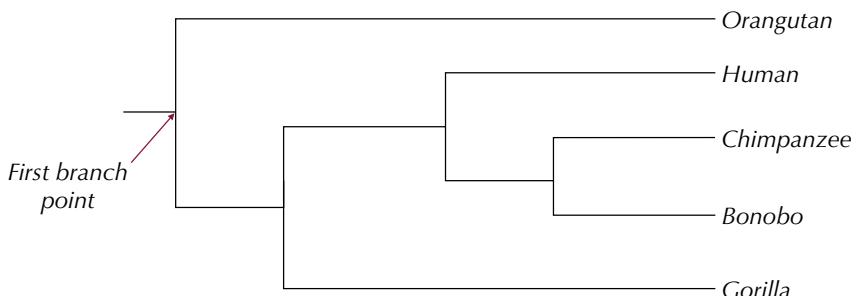


Figure 1: Phylogenetic tree of the Hominidae family.

This tree shows the relationship between members of the Hominidae family (great apes and humans). The first branch point represents a common ancestor of all the family members. This ancestor is now extinct. Orangutans were the first group to diverge (evolve to become a different species) from this common ancestor.

Each of the following branch points represents another common ancestor from which a different group diverged. Gorillas diverged next, then humans, closely followed by bonobos and chimpanzees.

Closely related species diverged away from each other most recently. E.g. humans and chimpanzees are closely related, as they diverged very recently. You can see this because their branches are close together.

Taxonomy

Taxonomy is the science of classification. It involves naming organisms and organising them into groups. This makes it easier to identify and study them. Scientists now take into account phylogeny when classifying organisms, and group organisms according to their evolutionary relationships.

There are eight levels of groups used to classify organisms. These groups are called **taxa**. Each group is called a taxon. The groups are arranged in a **hierarchy**, with the largest groups at the top and the smallest groups at the bottom. Organisms can only belong to one group at each level in the hierarchy — there's no overlap.

Organisms are first sorted into three large groups (or taxa) called domains — the Eukarya, Bacteria and Archaea. Related organisms in a domain are then sorted into slightly smaller groups called kingdoms, e.g. all animals are in the animal kingdom. More closely related organisms from that kingdom are then grouped into a phylum, then grouped into a class, and so on down the eight levels of the hierarchy. This is illustrated in Figure 3.

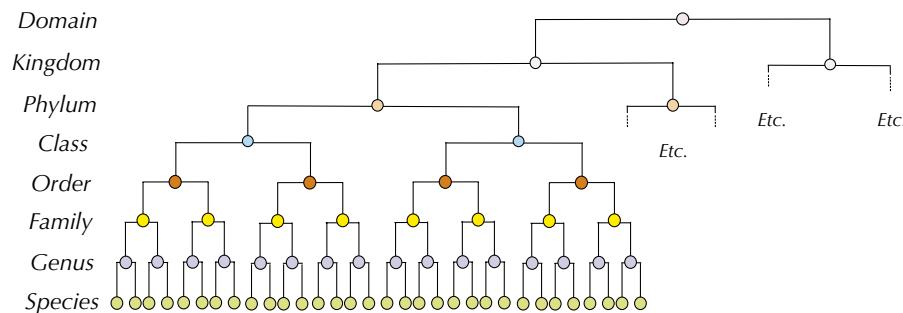


Figure 3: A diagram illustrating the taxonomic groups used in classification.

Exam Tip

You need to learn the names and order of the groups (taxa). If you're struggling to remember the order, try this mnemonic...

Demanding Kids Prefer Chips Over Floppy Green Spinach.

Example — the classification of humans

Domain = *Eukarya*, Kingdom = *Animalia*, Phylum = *Chordata*, Class = *Mammalia*, Order = *Primates*, Family = *Hominidae*, Genus = *Homo*, Species = *sapiens*.

As you move down the hierarchy, there are more groups at each level but fewer organisms in each group. The organisms in each group also become more closely related. The hierarchy ends with **species** — the groups that contain only one type of organism (e.g. humans). A species is a group of similar organisms able to reproduce to give fertile offspring.

Tip: You need to learn the definition of a species.

Example

If a female horse breeds with a male horse of the same species their offspring will be fertile. But if a female horse breeds with a male donkey their offspring (known as a mule) will be infertile. Because horses and donkeys can't reproduce to give fertile offspring, they're classified as separate species.

Scientists constantly update classification systems because of discoveries about new species and new evidence about known organisms (e.g. DNA sequence data — see page 243).

The binomial system

The nomenclature (naming system) used for classification is called the binomial system — all organisms are given one internationally accepted scientific name in Latin that has two parts.

The first part of the name is the genus name and has a capital letter. The second part is the species name and begins with a lower case letter. Names are always written in italics (or they're underlined if they're handwritten).

Tip: You'll often see the genus shortened to just the first letter. E.g. *E. coli* is short for *Escherichia coli* — *Escherichia* is the genus and *coli* is the species.

Examples

Humans are *Homo sapiens* — The genus is *Homo* and the species is *sapiens*. Dogs are *Canis familiaris* — The genus is *Canis* and the species is *familiaris*. Cats are *Felis catus* — The genus is *Felis* and the species is *catus*.

Exam Tip

The plural of genus is genera. You'll see this sometimes in exams.

Tip: Some species have the same genus name and species name, e.g. *Bison bison* (which is commonly known as a... erm, bison). Imaginative.

Tip: It might surprise you that some organisms are closely related — but just because you can't see a similarity in their features doesn't mean the phylogenetic tree is wrong.

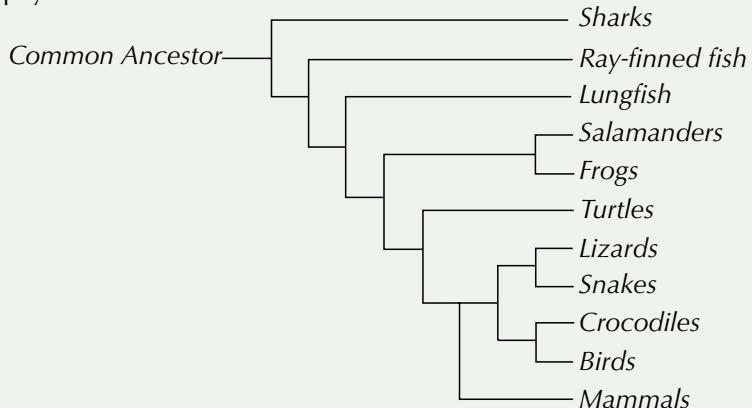
Giving organisms a scientific name enables scientists to communicate about organisms in a standard way, which helps to avoid the confusion of using common names.

Example

Americans call a type of bird cockatoos and Australians call them flaming galahs, but it's the same bird. If the correct scientific name is used — *Eolophus roseicapillus* — there's no confusion.

Practice Questions — Application

Q1 The diagram below shows a simplified phylogenetic tree for the phylum Chordata:



- a) Which group was first to diverge from the common ancestor?
- b) Are frogs more closely related to salamanders or turtles?
- c) To which other group are:
 - (i) birds most closely related?
 - (ii) snakes most closely related?

Q2 Donkeys are part of the phylum Chordata.

The binomial name for a donkey is *Equus asinus*.

Complete the table below for the classification of the donkey.

| Taxon | |
|---------|----------------|
| | Eukarya |
| Kingdom | Animalia |
| | |
| Class | Mammalia |
| Order | Perrisodactyla |
| Family | Equidae |
| | |
| | |

Practice Questions — Fact Recall

Q1 How does a phylogenetic classification system attempt to arrange organisms into groups?

Q2 What is the naming system used in biological classification called?

2. Classification Using Courtship Behaviour

Similar species don't only look similar — they also behave in a similar way. This means that behaviour can also be used to help classify species. Courtship behaviour can be particularly useful when classifying species.

What is courtship behaviour?

Courtship behaviour is carried out by organisms to attract a mate of the right species. It can be fairly simple or quite complex.

Examples

Simple Courtship behaviours:

- Releasing a chemical — e.g. male bumble bees produce chemicals called pheromones to attract female bumble bees to their territory.
- Using sound — e.g. male red deer make a roaring noise to attract a mate.
- Visual displays — e.g. the great tit will attract a mate by puffing out its chest to show off its black stripe.

Complex Courtship behaviours:

- Dancing — e.g. blue-footed boobies perform a complex dance which involves lifting up their feet to show off the blue colour (see Figure 1).
- Building — e.g. bowerbirds construct bowers (shelters) made of leaves, twigs, flowers, shells, stones and whatever else the male can find (see Figure 2).

Courtship behaviours can be performed by either the male or the female or may sometimes involve both sexes.

Using courtship behaviour to classify species

Courtship behaviour is species specific — only members of the same species will do and respond to that courtship behaviour. This allows members of the same species to recognise each other, preventing interbreeding and making reproduction more successful (as mating with the wrong species won't produce fertile offspring).

Examples

- Fireflies give off pulses of light. The pattern of flashes is specific to each species.
- Crickets make sounds that are similar to Morse code, the code being different for different species.
- Male peacocks show off their colourful tails. This tail pattern is only found in peacocks (see Figure 3).
- Male butterflies use chemicals to attract females. Only those of the correct species respond.

Because of this specificity, courtship behaviour can be used to classify organisms. The more closely related species are, the more similar their courtship behaviour.

Learning Objectives:

- Know that courtship behaviour is a necessary precursor to successful mating.
- Understand the role of courtship in species recognition.

Specification Reference 3.4.5



Figure 1: The dance of the blue-footed booby.



Figure 2: A male bowerbird constructing a bower.



Figure 3: A male peacock displaying his tail feathers.

Practice Questions — Application

Male fireflies give off pulses of light to attract females. To investigate this, 10 fireflies were caught and the pattern of light pulses that they used was observed. The table below shows the results:

Exam Tip

Whatever the topic, examiners love data-interpretation questions. Get used to seeing data presented in lots of different ways.

| Firefly | Pattern of pulses produced |
|---------|----------------------------|
| 1 | xx-xx-xx-xx |
| 2 | xxx-x-xxx-x-xxx |
| 3 | x-xx-xxx-x-xx-xxx |
| 4 | xxx-xxx-xxx |
| 5 | x-x-x-x-x-x |
| 6 | xx-xx-xx-xx |
| 7 | x-xx-x-xx-x-xx |
| 8 | xx-xx-xx-xx |
| 9 | xxx-xxx-xxx |
| 10 | x-xx-xxx-x-xx-xxx |

Key:

x = flash of light
- = no light

- Q1 What do the patterns of light pulses they produce suggest about fireflies 4 and 9?
- Q2 How many different species of firefly were caught in total?
- Q3 Are fireflies 1 and 3 likely to be closely related or distantly related? Explain your answer.
- Q4 Firefly 2 was later found to belong to a family of fireflies that all start their display with three pulses of light. Are any of the other fireflies likely to belong to the same family?

Practice Questions — Fact Recall

- Q1 What is courtship behaviour?
- Q2 Why can courtship behaviour be used to classify species?

3. Classification Using DNA or Proteins

Classifying organisms according to their evolutionary relationships (see page 238) isn't easy, but advances in DNA and molecular technology are helping us to classify organisms more accurately.

Clarifying evolutionary relationships

New or improved technologies can result in new discoveries being made and the relationships between organisms being clarified. This can lead to classification systems being updated. Technologies that have been useful for clarifying evolutionary relationships include:

Genome sequencing

Advances in genome sequencing have meant that the entire base sequence of an organism's DNA can be determined. The DNA base sequence of one organism can then be compared to the DNA base sequence of another organism, to see how closely related they are. Closely related species will have a higher percentage of similarity in their DNA base sequence, e.g. humans and chimps share around 94%, humans and mice share about 86%.

Learning Objectives:

- Appreciate that advances in genome sequencing and immunology help to clarify evolutionary relationships between organisms.
- Be able to interpret data relating to similarities and differences in the base sequences of DNA and in the amino acid sequences of proteins to suggest relationships between different organisms within a species and between species.

Specification Reference 3.4.5 and 3.4.7

Example

Genome sequencing has clarified the relationship between skunks and members of the Mustelidae family (e.g. weasels and badgers). Skunks were classified in the Mustelidae family until their DNA sequence was revealed to be significantly different to other members of that family. So they were reclassified into the family Mephitidae.

Tip: See page 204 for more on the DNA base sequence.

Comparing amino acid sequence

Proteins are made of amino acids. The sequence of amino acids in a protein is coded for by the base sequence in DNA (see p. 204). Related organisms have similar DNA sequences and so similar amino acid sequences in their proteins.

Example

Cytochrome C is a short protein found in many species. The more similar the amino acid sequence of cytochrome C in two different species, the more closely related the species are likely to be.

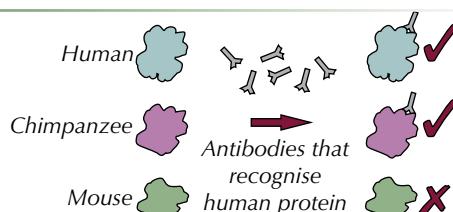
Tip: See page 119 for more on antibodies binding to proteins.

Immunological comparisons

Similar proteins will also bind the same antibodies.

Example

If antibodies to a human version of a protein are added to isolated samples from some other species, any protein that's like the human version will also be recognised (bound) by that antibody.



Tip: Proteins that bind antibodies will often form a precipitate (solid mass) in solution. The more antibodies the protein binds, the more precipitate will form — so the amount of precipitate can be used to determine how similar two proteins are.

Interpreting data

You might be given data on DNA and protein similarities to interpret in your exam. Don't panic. Just look at what's in front of you and think logically.

Here are a few examples of the type of thing you might get:

Example 1

The table below shows the % similarity of DNA using DNA sequence analysis between several species of bacteria.

Species A and B have a higher percentage of DNA in common with each other than they do with either species C or D. This means that A and B are more closely related to each other than they are to either C or D.

| | Species A | Species B | Species C | Species D |
|-----------|-----------|-----------|-----------|-----------|
| Species A | 100% | 86% | 42% | 44% |
| Species B | 86% | 100% | 51% | 53% |
| Species C | 42% | 51% | 100% | 49% |
| Species D | 44% | 53% | 49% | 100% |

Tip: You can also compare DNA base sequences to determine relationships between organisms of the same species. Again, the more similarities there are, the more closely related the two organisms are likely to be.

Example 2

The diagram below shows the DNA sequence for gene X in three different species.

Species A: ATTGTCTGATTGGTGCTAGTCGTCGATGCTAGGATCG

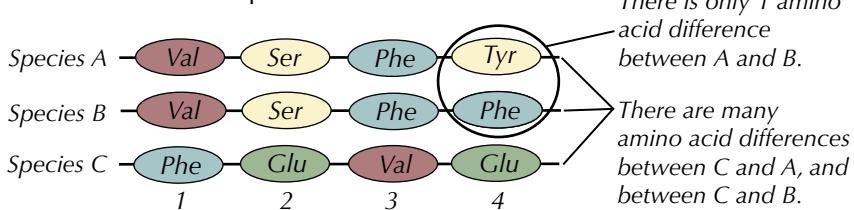
Species B: ATTGTATGATTGGTGCTAGTCGCGATGCTAGGATCG

Species C: ATTGATTGAAAGGAGCTACTCGTAGATATAAGGAGGT

There are 13 differences between the base sequences in species A and C, but only 2 differences between the base sequences in species A and B. This suggests that species A and B are more closely related than A and C.

Example 3

The diagram below shows the amino acid sequences of a certain protein from three different species.



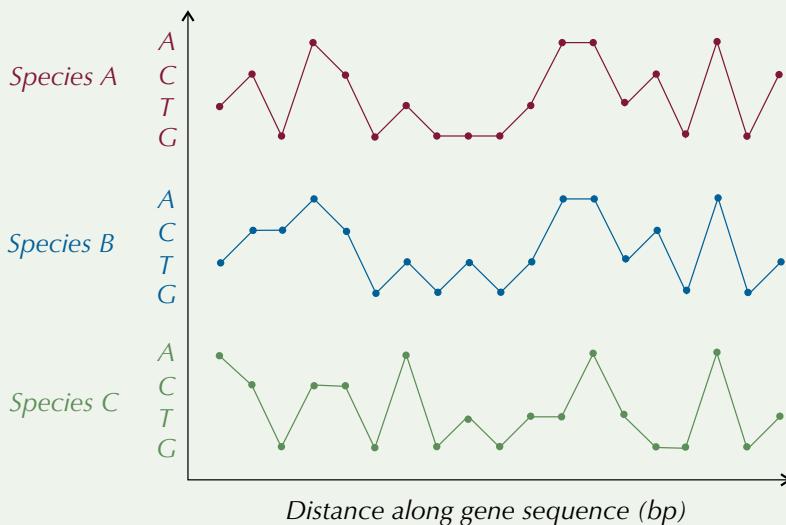
Tip: 'Val', 'Ser', 'Phe' and 'Glu' are short for the names of the amino acids.

You can see that the amino acid sequences from species A and B are very similar. The sequence from species C is very different to any of the other sequences. This suggests that species A and B are more closely related.

Practice Questions — Application

- Q1 The amino acid sequence of the insulin protein was determined for humans, horses and chickens. When this was done, it was found that horse insulin was more similar to human insulin than chicken insulin. What do these results suggest?

Q2 The graph below illustrates the sequence of a small stretch of DNA in 3 different species:



- a) Using the graph, write down the base sequence for this stretch of DNA in each of the three species.
 - b) In how many places do the base sequences of species A and B differ?
 - c) In how many places do the base sequences of species A and C differ?
 - d) Is species A more closely related to species B or species C? Explain your answer.
 - e) To which of the two species is species C most closely related? Explain your answer.

Q3 Antibodies against a protein from species X were fluorescently labelled and mixed with cells from three other species. Any unbound antibody was then washed away and the level of remaining fluorescence was recorded. Use the results table above to determine which species is most closely related to species X. Explain your choice.

| Species | Relative fluorescence after washing |
|---------|-------------------------------------|
| A | 0.2 |
| B | 10.5 |
| C | 2.1 |

| Species | Relative fluorescence after washing |
|---------|--|
| A | 0.2 |
| B | 10.5 |
| C | 2.1 |

Tip: The distance along the gene sequence on the diagram is given as 'bp' — base pairs.

Tip: Antibodies are often linked to a fluorescent protein (e.g. GFP). This allows the antibodies to be detected once they have bound to a protein.

Learning Objectives:

- Know that estimates of genetic diversity within, or between species, can be made by comparing:
 - the frequency of measurable or observable characteristics
 - the base sequence of DNA
 - the base sequence of mRNA
 - the amino acid sequence of the proteins encoded by DNA and mRNA.
- Be able to appreciate that gene technology has caused a change in the methods of investigating genetic diversity — inferring DNA differences from measurable or observable characteristics has been replaced by direct investigation of DNA sequences.

Specification Reference 3.4.7

Tip: DNA is copied into mRNA in order to make a protein — see pages 207-208.

4. Using Gene Technologies to Assess Genetic Diversity

Gene technologies haven't just helped in the clarification of evolutionary relationships, they've also changed how genetic diversity is assessed.

How are gene technologies used to assess genetic diversity?

You might remember from page 226, that genetic diversity is the number of different alleles in a population.

Early estimates of genetic diversity were made by looking at the frequency of measurable or observable characteristics in a population, e.g. the number of different eye colours in a population and the number of people with each particular eye colour. Since different alleles determine different characteristics (see page 226) a wide variety of each characteristic in a population indicates a high number of different alleles — and so a high genetic diversity. However gene technologies have now been developed that allow us to measure genetic diversity directly:

Examples

- Different alleles of the same gene will have slightly different DNA base sequences. Comparing the DNA base sequences of the same gene in different organisms in a population allows scientists to find out how many alleles of that gene there are in that population.
- Different alleles will also produce slightly different mRNA base sequences, and may produce proteins with slightly different amino acid sequences, so these can also be compared.

These new technologies can be used to give more accurate estimates of genetic diversity within a population (or species) than can be made just by looking at the frequency of observable characteristics. They also allow the genetic diversity of different species to be compared more easily.

Practice Question — Application

- Q1 The colour and pattern of the shells of the snail species *Cepaea nemoralis* is controlled by several genes, each of which has several alleles. A scientist interested in the species thinks that there may be more genetic diversity in the genes controlling shell colour and pattern in populations of snails from warmer climates, than in populations of snails from colder climates.
- Describe and explain how comparing the DNA sequences of individuals from different populations could be used to test this theory.
 - Before gene technologies like this became available, how was genetic diversity estimated?

Practice Question — Fact Recall

- Q1 What are the advantages of using gene technologies rather than traditional methods to assess genetic diversity in a population?

5. Investigating Variation

Sometimes it's helpful to know whether variations in a population are primarily due to genetics or the environment. The next few pages are all about how variation is studied and how data on variation is analysed.

Causes of variation

Variation is the differences that exists between individuals. There's variation between species and within species.

Variation can be caused by genetic factors. Different species have different genes, which causes variation between species. Individuals of the same species have the same genes, but different alleles (versions of genes) — this causes variation within a species.

Variation within a species can also be caused by differences in the environment, e.g. climate, food, lifestyle.

Most variation within a species is caused by a combination of genetic and environmental factors. E.g. genes determine how tall an organism can grow, but nutrient availability affects how tall the organism actually grows.

Learning Objectives:

- Know that quantitative investigations of variation within a species involve:
 - collecting data from random samples
 - calculating a mean value of the collected data and the standard deviation of that mean
 - interpreting mean values and their standard deviations.

Specification Reference 3.4.7

Tip: Variation between species is known as interspecific variation. Variation within a species is known as intraspecific variation.

Population samples

When studying variation within a species, you usually only look at a sample of the population, not the whole thing. For most species it would be too time-consuming or impossible to catch all the individuals in the group. So samples are used as models for the whole population.

Random sampling

Because sample data will be used to draw conclusions about the whole population, it's important that it accurately represents the whole population and that any patterns observed are tested to make sure they're not due to chance.

To make sure the sample isn't **biased**, it should be random.

For example, if you were looking at plant species in a field you could pick random sample sites by dividing the field into a grid and using a random number generator to select coordinates — see Figure 1.

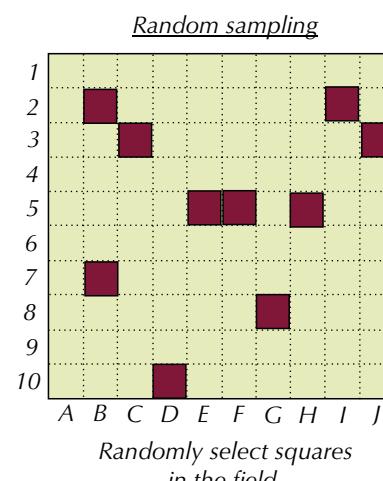
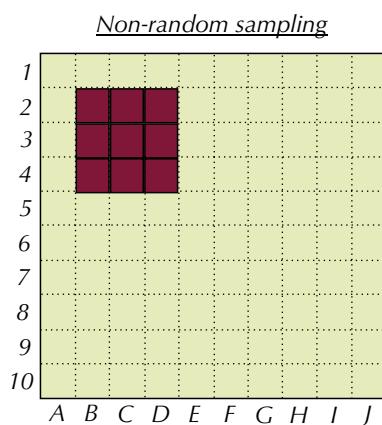


Figure 1: Diagram to show non-random sampling by picking a small area (left) and random sampling using a random number generator (right).

Tip: A sample is biased if it doesn't represent the population as a whole. For example, if you were looking at the average height of students in a school but only measured the heights of people from one particular class, the sample would be biased.

Tip: A random number generator will give you coordinates at random, e.g. C3, E5, etc. Then you just take your samples from these coordinates.

To ensure any variation observed in the sample isn't just due to chance, it's important to analyse the results statistically. This allows you to be more confident that the results are true and therefore will reflect what's going on in the whole population. There's more on statistical analysis on pages 8-9.

Mean and standard deviation

You can use the mean and standard deviation to measure how much variation there is in a sample.

Mean

The mean is an average of the values collected in a sample. Find it using this formula:

$$\text{mean} = \frac{\text{total of all the values in your data}}{\text{the number of values in your data}}$$

Tip: When you calculate the mean, check that it's within the range of values that you used in the calculation. If the mean isn't within the range, you know you've calculated it wrong. E.g. the mean here should be between 4 and 9 cm.

Example — Maths Skills

The heights of different seedlings in a group are: 6 cm, 4 cm, 7 cm, 6 cm, 5 cm, 8 cm, 7 cm, 5 cm, 7 cm and 9 cm.

To calculate the mean, add all of the heights together and divide by the number of seedlings:

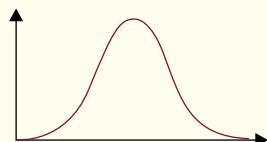
$$\begin{aligned}\text{Mean height} &= (6 + 4 + 7 + 6 + 5 + 8 + 7 + 5 + 7 + 9) \div 10 = 64 \div 10 \\ &= 6.4 \text{ cm}\end{aligned}$$

The mean can be used to tell if there is variation between samples.

Examples

- The mean height of a species of tree in woodland A = 26 m, woodland B = 32 m and woodland C = 35 m. So the mean height varies.
- The mean number of leaves on a clover plant in field X = 3, field Y = 3 and field Z = 3. So the mean number of leaves does not vary.

Tip:
Normal distribution (symmetrical):



Not a normal distribution (skewed):



Most samples will include values either side of the mean, so you end up with a bell-shaped graph — this is called a **normal distribution** (see Figure 2). A normal distribution is symmetrical about the mean.

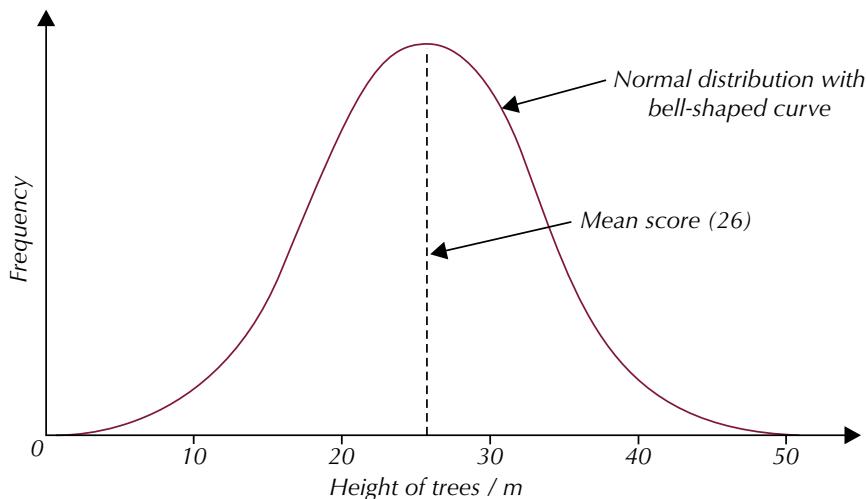
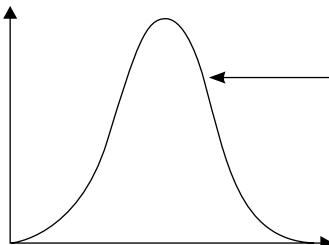


Figure 2: The height of trees in woodland A.

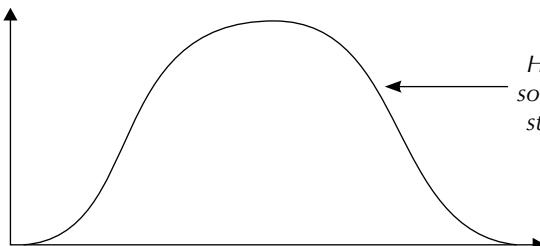
Standard deviation

The standard deviation tells you how much the values in a single sample vary. It's a measure of the spread of values about the mean. Sometimes you'll see the mean written as, for example, 9 ± 3 . This means that the mean is 9 and the standard deviation is 3, so most of the values are spread between 6 and 12.

Both of the graphs in Figure 3 show a normal distribution. However, the values in a sample can vary a little or a lot:



Here, all the values are similar and close to the mean — the data varies little, so the graph is steep and the standard deviation is small.

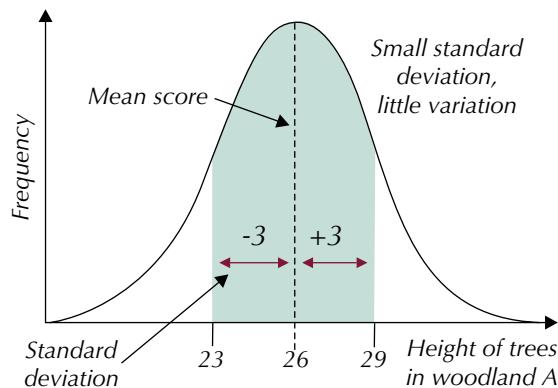


Here, the values vary a lot, so the graph is fatter and the standard deviation is large.

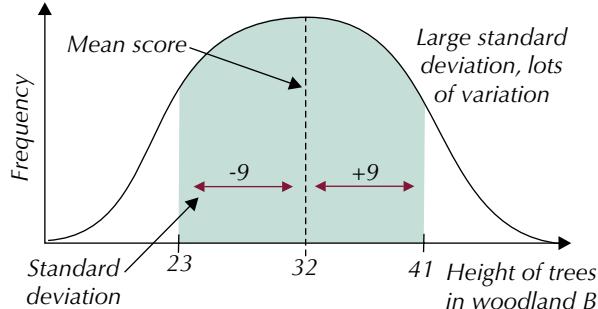
Figure 3: A normal distribution curve with a small standard deviation (top) and with a large standard deviation (bottom).

Example

Height of trees in woodland A:
mean = 26,
standard deviation = 3



Height of trees in woodland B:
mean = 32,
standard deviation = 9



So the trees are generally taller in woodland B but there's a greater variation in height, compared to woodland A.

Exam Tip

You won't get marks for describing the standard deviation as the spread of results — it's the spread of values about the mean.

Exam Tip

You need to know how to interpret data that includes standard deviations for your exam.

Tip: Values with a larger standard deviation show greater variation.

Calculating standard deviation

Figure 4 shows the formula for finding the standard deviation of a group of values:

$$S = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

This symbol is sigma
— it means 'sum of.'

'S' just stands for standard deviation.

Square root sign

'x' stands for a value in the data set, and ' \bar{x} ' is the mean. So ' $(x - \bar{x})^2$ ' means "take away the mean from the value, then square the result."

'n' stands for the number of values.

Figure 4: Explanation of the formula for standard deviation.

Exam Tip

You won't be asked to calculate standard deviation in your written exams, but it's helpful to know how to do it for when you're analysing data in class or doing practical investigations.

Tip: Standard deviation (S) can also be represented by the Greek letter sigma: ' σ '.

Example — Maths Skills

The table shows the height of four different trees in a forest.

To find the standard deviation:

- Write out the equation:
$$S = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$
- Work out the mean height of the trees, \bar{x} :
$$(22 + 27 + 26 + 29) \div 4 = 26$$
- Work out $(x - \bar{x})^2$ for each value of x . For each tree height in the table, you need to take away the mean, then square the answer:

| | |
|--------------------------------|----------------------------|
| A: $(22 - 26)^2 = (-4)^2 = 16$ | B: $(27 - 26)^2 = 1^2 = 1$ |
| C: $(26 - 26)^2 = 0^2 = 0$ | D: $(29 - 26)^2 = 3^2 = 9$ |
- Add up all these numbers to find $\sum (x - \bar{x})^2$:
$$16 + 1 + 0 + 9 = 26$$
- Divide this number by the number of values, n , minus 1. Then take the square root to get the answer:
$$26 \div 3 = 8.66\dots$$

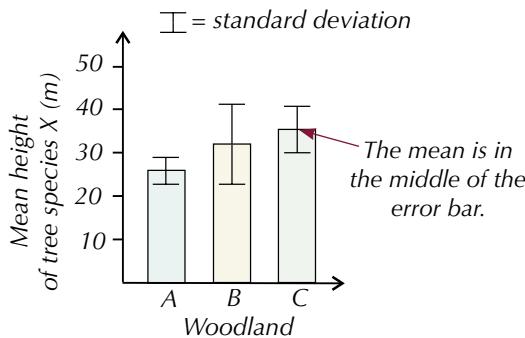
$$\sqrt{8.66\dots} = 2.9 \text{ to 2 s.f.}$$

| Tree | Height (m) |
|------|------------|
| A | 22 |
| B | 27 |
| C | 26 |
| D | 29 |

Standard deviation is one method of calculating the dispersion of data. Another method of calculating dispersion is by looking at the range — see page 5. This is simply the difference between the highest and lowest figures in the data. Standard deviation is more useful than the range because it takes into account all the values in the data set, whereas the range only uses two. This makes the range more likely to be affected by an anomalous result (an unusually high or low value in the data set) than standard deviation.

Using standard deviation to draw error bars

Standard deviations can be plotted on a graph or chart of mean values using **error bars**. Error bars extend one standard deviation above and one standard deviation below the mean (so the total length of an error bar is twice the standard deviation). The longer the bar, the larger the standard deviation and the more spread out the sample data is from the mean — see Figure 5.

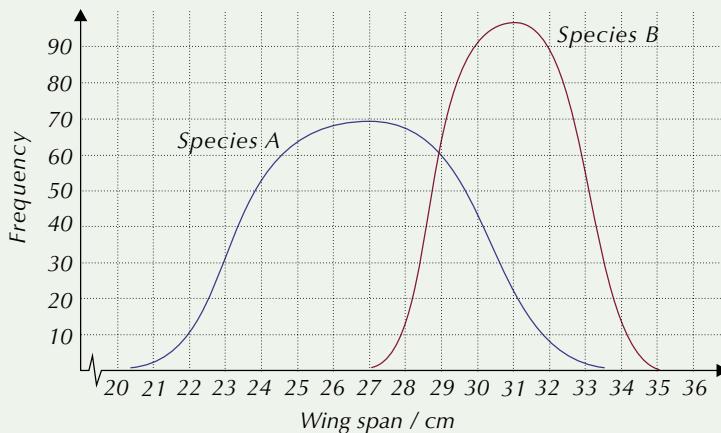


Tip: The smaller the error bars, the smaller the standard deviation and the less the data in the sample varies.

Figure 5: An example of a graph with standard deviation error bars.

Practice Questions — Application

- Q1 The graph below shows the wing spans of two different species of bird, both of which live in the same area of woodland.



- a) i) Describe the data.
ii) Which species shows a greater variation in wing span?
Explain your answer.
- b) How much longer is species B's mean wing span than species A's?
Give your answer as a percentage.

- Q2 The table on the right shows the length of five rainbow boa snakes measured by conservationists investigating the effect of habitat loss on the well-being of the species. Using the formula below, calculate the standard deviation of this data.

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

| Snake | length (cm) |
|-------|-------------|
| A | 177 |
| B | 182 |
| C | 190 |
| D | 187 |
| E | 191 |

Exam Tip

Always show your working in calculation questions. You could pick up marks for using the correct method, even if your final answer is wrong.

Practice Questions — Fact Recall

- Q1 How do you calculate the mean?
- Q2 What shape is the graph of a data set with a normal distribution?
- Q3 What does standard deviation measure?

Learning Objectives:

- Know that biodiversity can relate to a range of habitats, from a small local habitat to the Earth.
- Understand that species richness is a measure of the number of different species in a community.
- Know that an index of diversity describes the relationship between the number of species in a community and the number of individuals in each species.
- Be able to calculate an index of diversity (d) from the formula:

$$d = \frac{N(N - 1)}{\sum n(n - 1)}$$

where N = total number of organisms of all species and n = total number of organisms of each species.

Specification Reference 3.4.6

Tip: A species is a group of similar organisms able to reproduce to give fertile offspring (see page 239).

Tip: The number of species in a community and the abundance of each species is also known as the species diversity.

6. Biodiversity

Biodiversity is important — the higher the biodiversity in an ecosystem, the healthier that ecosystem is.

Terms you need to know

Before you can sink your teeth into the real meat of biodiversity, there are a few definitions you need to know:

- **Biodiversity** is the variety of living organisms in an area.
- A **habitat** is the place where an organism lives, e.g. a rocky shore or field.
- A **community** is all the populations of different species in a habitat.

Levels of biodiversity

Biodiversity can be considered at a range of scales from the local to the global:

- Local biodiversity — you could consider the variety of different species living in a small habitat that's local to you, e.g. a pond or meadow, or even your back garden. Some habitats will be more biodiverse than others.
- Global biodiversity — you could also consider the variety of species on Earth. Recent estimates put the total number of species on Earth at about 8.7 million. Biodiversity varies in different parts of the world — it is greatest at the equator and decreases towards the poles.

Measuring biodiversity

Biodiversity can be measured using species richness or an index of diversity.

Species richness is a measure of the number of different species in a community — which makes it a simple measure of biodiversity. It can be worked out by taking random samples of a community (see page 247) and counting the number of different species.

However, the number of different species in a community isn't the only thing that affects biodiversity. The population sizes of those species do too. Species that are in a community in very small numbers shouldn't be treated the same as those with bigger populations. This is where an index of diversity comes in.

An **index of diversity** is another way of measuring biodiversity. It's calculated using an equation that takes both the number of species in a community (species richness) and the abundance of each species (population sizes) into account.

You can calculate an index of diversity (d) using this formula:

$$d = \frac{N(N - 1)}{\sum n(n - 1)}$$

Where...
 N = Total number of organisms of all species
 n = Total number of organisms of one species
 Σ = 'Sum of' (i.e. added together)

The higher the number, the more diverse the area is. If all the individuals are of the same species (i.e. no biodiversity) the index is 1.

Example — Maths Skills

There are 3 different species of flower in this field — a red species, a white and a blue. There are 3 of the red species, 5 of the white and 3 of the blue.

There are 11 organisms altogether, so $N = 11$.

So the species diversity index of this field is:



Tip: When calculating the bottom half of the equation you need to work out the $n(n-1)$ bit for each different species then add them all together.

Example — Maths Skills

A student investigates the diversity of fish species in her local pond. She finds 46 fish of 6 different species. To help her calculate the index of diversity for the pond she draws the following table:

| Species | n (total number of organisms in species) | $n - 1$ | $n(n - 1)$ |
|--|--|---------|------------|
| A | 1 | 0 | 0 |
| B | 6 | 5 | 30 |
| C | 2 | 1 | 2 |
| D | 15 | 14 | 210 |
| E | 3 | 2 | 6 |
| F | 19 | 18 | 342 |
| N (total number of all organisms) | 46 | | |

She then uses the numbers from the table to calculate the diversity index:

$$d = \frac{N(N - 1)}{\sum n(n-1)} = \frac{46(46 - 1)}{0 + 30 + 2 + 210 + 6 + 342} = \frac{2070}{590} = 3.51 \text{ (3 s.f.)}$$

Tip: If you've got a lot of data, you might find it easier to plug the numbers into a table (as in example 2) to make sure you don't miss any steps.

Tip: $n(n - 1)$ just means $n \times (n - 1)$.

Tip: To get the figures in the last column in the table, you multiply together the figures in the second and third columns.

Practice Questions — Application

Q1 The table below shows the number of individuals of each species of insect found in two ponds.

| Species | Number of individuals found in Pond A | Number of individuals found in Pond B |
|---------------|--|--|
| Damselfly | 3 | 13 |
| Dragonfly | 5 | 5 |
| Stonefly | 2 | 7 |
| Water boatman | 3 | 2 |
| Crane fly | 1 | 18 |
| Pond skater | 4 | 9 |

Tip: This question continues on the next page.

Tip: This question is continued from the previous page.

Exam Tip

You can use a calculator to help you with this question — you'll be allowed to take one into the exam with you.

Exam Tip

Don't be thrown if you get asked to calculate species diversity using data from a graph — just read off the values carefully.

- a) What is the species richness of insects in Pond A?
b) Use the data provided in the table and the formula given below to calculate the index of diversity for:

i) Pond A

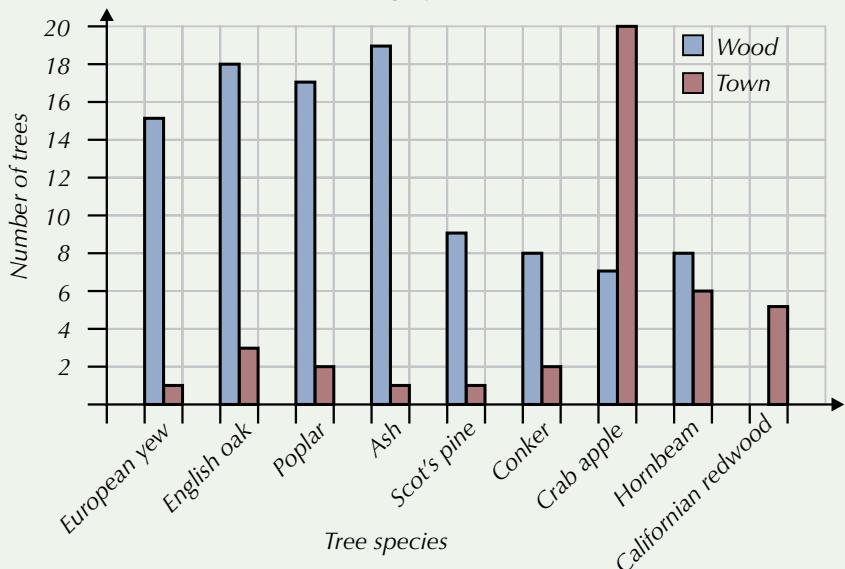
ii) Pond B

$$d = \frac{N(N - 1)}{\sum n(n-1)}$$

where N = total number of all organisms
and n = total number of organisms in one species.

- c) Birds and amphibians feed on insects. Which of the two ponds would you expect to have a higher diversity of birds and amphibians? Explain your answer.

- Q2 A study was conducted on the trees found in a wood and town. The results are shown in the graph below.



Exam Tip

If you've got time at the end of the exam, always go back over any calculation questions and check the answer — it's easy to make a silly mistake somewhere and lose marks.

- a) Use the data in the graph and the formula provided below to calculate the index of diversity for the tree species in:

i) the wood

ii) the town

$$d = \frac{N(N - 1)}{\sum n(n-1)}$$

where N = total number of all organisms
and n = total number of organisms in one species.

- b) The index of diversity gives a better estimate of the diversity of tree species than simply counting the number of species present. Explain why, using data from the graph to support your answer.

Practice Questions — Fact Recall

- Q1 What is the definition of biodiversity?

- Q2 What is a community?

7. Agriculture and Biodiversity

Lots of things humans do can affect biodiversity — including agriculture.

The impact of agriculture on biodiversity

Farmers try to maximise the amount of food they can produce from a given area of land. But many of the methods they use reduce biodiversity:

- Woodland clearance is done to increase the area of farmland. It directly reduces the number of trees and sometimes the number of different tree species. It also destroys habitats, so some species could lose their shelter and food source. This means that species will die or be forced to migrate to another suitable area, further reducing biodiversity.
- Hedgerow removal is also done to increase the area of farmland by turning lots of small fields into fewer large fields. This reduces biodiversity for the same reasons as woodland clearance.
- Pesticides are chemicals that kill organisms (pests) that feed on crops. This reduces diversity by directly killing the pests. Also, any species that feed on the pests will lose a food source, so their numbers could decrease too.
- Herbicides are chemicals that kill unwanted plants (weeds). This reduces plant diversity and could reduce the number of organisms that feed on the weeds.
- Monoculture is when farmers have fields containing only one type of plant (see Figure 2). A single type of plant reduces biodiversity directly and will support fewer organisms (e.g. as a habitat or food source), which further reduces biodiversity.

Learning Objectives:

- Know that farming techniques reduce biodiversity.
- Understand the need for a balance between conservation and farming.

Specification Reference 3.4.6



Figure 1: Spraying herbicide on crops.



Figure 2: A monoculture field containing a single crop.

Conservation schemes

Agriculture is one way of producing the resources we need from the environment — we need it to produce food and fibres for clothing, as well as some medicines and fuels. Biodiversity helps maintain the environment. It provides us with new sources of food and medicines, and it benefits agriculture, e.g. a wide variety of insects help to pollinate crops. So there needs to be a balance between agriculture and biodiversity. Conservationists try to achieve this through **conservation schemes**.

Examples

- Giving legal protection to endangered species.
- Creating protected areas such as SSSIs (Sites of Special Scientific Interest) and AONBs (Areas of Outstanding Natural Beauty). These restrict further development, including agricultural development.
- The Environmental Stewardship Scheme encourages farmers to conserve biodiversity, e.g. by replanting hedgerows and leaving margins around fields for wild flowers to grow.

Tip: We need to make sure we produce enough food to feed our growing population, as well as conserving biodiversity.

Analysing the effect of agriculture on biodiversity

Exam Tip

Don't worry — you won't be expected to calculate a correlation coefficient in your written exams. You are expected to know when to use this type of test for statistical analysis though, and you could be asked to interpret the results of the test — see next page for more.

Tip: A positive correlation means that as one variable increases, so does the other. A negative correlation means that as one variable increases, the other decreases. See page 15 for more.

Tip: A data pair consists of the two corresponding figures for each variable. E.g. 0 pesticide applications and an index of diversity of 4.89 make up one data pair.

If you need to work out whether there's a correlation between two variables or not (or how strongly two variables are correlated), you can calculate the **Spearman's rank correlation coefficient (r_s)**. This is a type of correlation coefficient and an example of a statistical test (see page 9). It uses the formula:

$$r_s = 1 - \frac{6\sum d^2}{n(n^2 - 1)}$$

where ' d ' is the 'difference in rank between data pairs' and ' n ' is the total number of data pairs

The result of the test is a number between -1 and +1. If the figure is -1, then there is a perfect negative correlation between the two variables. If the figure is +1, then there's a perfect positive correlation. The closer the figure is to 0, the weaker the correlation is. It'll all become clear with an example...

Example — Maths Skills

A team of biologists investigated the effect of repeated pesticide applications on the number and abundance of different insect species found on an area of crops. They applied pesticides once a month over a 6 month period. They measured the index of diversity for the test area seven days after each pesticide application. The table on the right shows their results.

| Number of pesticide applications | Index of Diversity |
|----------------------------------|--------------------|
| 0 | 4.89 |
| 1 | 4.19 |
| 2 | 3.80 |
| 3 | 3.12 |
| 4 | 3.26 |
| 5 | 2.36 |
| 6 | 1.92 |

- First, rank both sets of data, keeping the data pairs together. The highest value for each variable is given the rank of 1, the second highest value is ranked 2, etc.

| Number of pesticide applications | Rank | Index of Diversity | Rank |
|----------------------------------|------|--------------------|------|
| 0 | 7 | 4.89 | 1 |
| 1 | 6 | 4.19 | 2 |
| 2 | 5 | 3.80 | 3 |
| 3 | 4 | 3.12 | 5 |
| 4 | 3 | 3.26 | 4 |
| 5 | 2 | 2.36 | 6 |
| 6 | 1 | 1.92 | 7 |

- Then work out the difference in rank between the two values in each data pair (d) and square it to calculate d^2 .

| Number of pesticide applications | Rank | Index of Diversity | Rank | Difference between ranks (d) | d^2 |
|----------------------------------|------|--------------------|------|----------------------------------|-------|
| 0 | 7 | 4.89 | 1 | 6 | 36 |
| 1 | 6 | 4.19 | 2 | 4 | 16 |
| 2 | 5 | 3.80 | 3 | 2 | 4 |
| 3 | 4 | 3.12 | 5 | 1 | 1 |
| 4 | 3 | 3.26 | 4 | 1 | 1 |
| 5 | 2 | 2.36 | 6 | 4 | 16 |
| 6 | 1 | 1.92 | 7 | 6 | 36 |

- Now count the number of data pairs (n). There are 7 data pairs here, so $n = 7$.
- Now you can put all this information into the Spearman's rank formula:

$$\begin{aligned}
 r_s &= 1 - \frac{6\sum d^2}{n(n^2 - 1)} = 1 - \frac{6(36 + 16 + 4 + 1 + 1 + 16 + 36)}{7(7^2 - 1)} \\
 &= 1 - \frac{6 \times 110}{7 \times 48} = 1 - \frac{660}{336} \\
 &= \mathbf{-0.964} \text{ (3 s.f.)}
 \end{aligned}$$

Because the figure is negative and close to -1, this suggests that there is a strong negative correlation between the number of applications of pesticides and the diversity index.

Once you've got your result, you need to find out if it's statistically significant or not. First, you need to come up with a **null hypothesis**. When you're investigating correlations, the null hypothesis should always be that there is no correlation between the factors you're investigating — even if you expect that there will be. So the null hypothesis for the example above could be "there is no correlation between the number of applications of pesticides over a 6 month period and the biodiversity of the test area".

The result of the Spearman's rank test allows you to decide whether the null hypothesis can be rejected. To determine whether the null hypothesis can be rejected, you consult a table of **critical values** (see Figure 3).

The result is compared to the critical value at $p = 0.05$, which corresponds to n for the data you're looking at (in this case, 7). This value represents the point at which the correlation you're investigating would occur 95 out of 100 times, so there's only a 5% chance that the correlation is down to chance. You can reject the null hypothesis if the result of your test is higher than this value. If your result is a negative number, you ignore the minus sign when comparing it to the critical value. In this example, the Spearman's rank correlation coefficient (0.964) is higher than the relevant critical value, so the null hypothesis can be rejected. The result is statistically significant and the positive correlation is unlikely to be due to chance.

Tip: You usually come up with the null hypothesis before you start your investigation — but we've explained how to calculate the correlation coefficient first here for clarity.

| <i>n</i> | <i>p = 0.05</i> |
|-----------------|------------------------|
| 7 | 0.786 |
| 8 | 0.738 |
| 9 | 0.700 |
| 10 | 0.648 |
| 11 | 0.618 |
| 12 | 0.587 |

Figure 3: A table of critical values for the Spearman's rank test.

Tip: When you're checking your result against the critical value, always make sure that you use the right critical value for the number of data pairs that you've investigated.

Tip: If your result is not statistically significant, it means it could just be down to chance.

Practice Question — Application

Q1 A group of scientists were investigating the effect of the number of different types of crop being grown in a field on the field's biodiversity. They calculated the index of diversity for 8 different 0.5 km² fields, each with a different number of types of crop growing in them. Their results are shown in the table on the right.

| Number of Crop Types | Index of Diversity |
|----------------------|--------------------|
| 1 | 1.87 |
| 2 | 2.24 |
| 3 | 2.71 |
| 4 | 3.18 |
| 5 | 4.01 |
| 6 | 3.59 |
| 7 | 4.44 |
| 8 | 4.97 |

$$r_s = 1 - \frac{6\sum d^2}{n(n^2 - 1)}$$

- Using the formula on the right, calculate the Spearman's rank correlation coefficient for this data.
- Does the result suggest a positive or negative correlation?
- Using the table of critical values in Figure 3 above, determine whether the null hypothesis "there is no correlation between the number of different types of crop growing in a field and its biodiversity" should be accepted or rejected.

Practice Questions — Fact Recall

- Q1 Many farmers clear woodland and remove hedgerows from their land.
Explain:
- why this is done.
 - how these practices can reduce biodiversity.
- Q2 Why does there need to be a balance between agriculture and conservation?

Section Summary

Make sure you know...

- That scientists take into account phylogeny (the evolutionary history and relationships of organisms) when classifying organisms.
- That the classification system consists of a hierarchy — larger groups are divided into smaller groups, and there is no overlap between groups.
- The eight levels of groups (taxa) used to classify organisms — domain, kingdom, phylum, class, order, family, genus and species.
- That a species is defined as a group of similar organisms able to reproduce to give fertile offspring.
- That every organism is given a two-part scientific name using the binomial naming system. The first part of the name is the genus the organism belongs to and the second part is the species. This naming system allows every organism to be universally identified.
- That courtship behaviour is carried out to attract a mate of the right species.
- That courtship behaviour is species specific, so it can be used to help classify organisms.
- That advances in genome sequencing and immunology have helped clarify evolutionary relationships between organisms.
- How to interpret data on DNA or protein similarities and use this to suggest relationships between different organisms.
- How early estimates of genetic diversity were made by comparing the number of different observable characteristics in a population.
- That the use of gene technologies has changed the way we investigate genetic diversity and estimates can now be made by comparing the base sequence of DNA, the base sequence of mRNA or the amino acid sequence of the proteins encoded by DNA and mRNA.
- How variation can be investigated using random sampling, mean values and standard deviations.
- How to interpret mean values and standard deviations.
- That biodiversity can be considered on a range of scales — from local to global.
- That species richness is a measure of the number of different species in a community and so is a simple measure of biodiversity.
- That an index of diversity is also a measure of biodiversity and that it takes into account both species richness and population size.
- How to calculate an index of diversity from given data.
- How farming can reduce biodiversity (e.g. through woodland clearance, pesticides, herbicides, hedgerow removal, competition and monoculture).
- Why it's important that there's a balance between agriculture and conservation.
- When to use a correlation coefficient (Spearman's rank) and how to compare the result to a table of critical values to determine its significance.

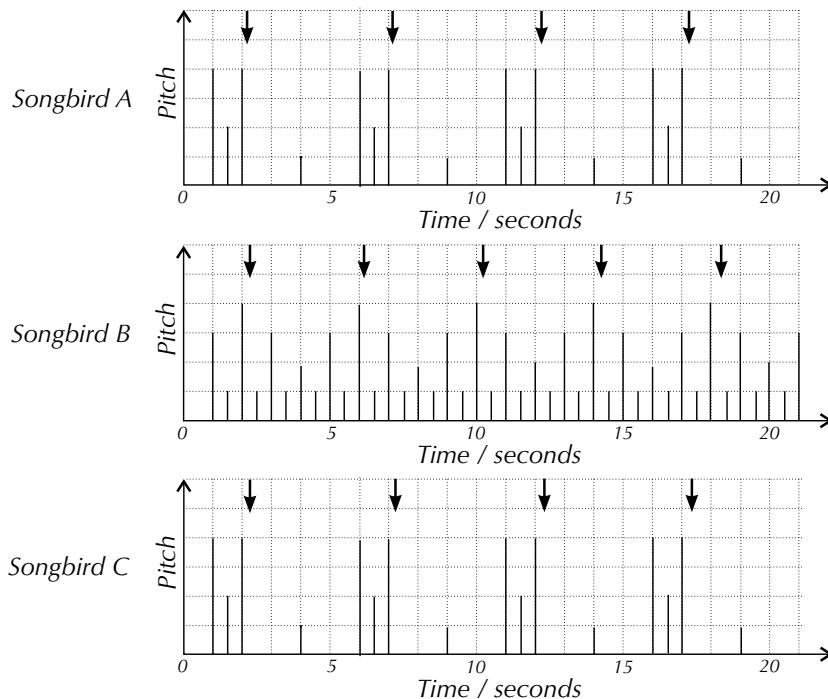
Exam-style Questions

- 1 Songbirds use elaborate songs to attract a mate.
This is a type of courtship behaviour.
- 1.1 Explain **one** way in which courtship behaviour makes organisms more likely to mate successfully.

(2 marks)

The graphs in **Figure 1** illustrate a mating song from three different songbirds. The arrows on the graphs show the beginnings of each phrase. Each phrase is made up of a series of notes and is repeated multiple times to make a song:

Figure 1



- 1.2 Calculate the number of phrases sung each minute by songbird B.
- (1 mark)
- 1.3 What can you conclude about the classification of songbird A and songbird C from the data in **Figure 1**? Explain your answer.
- (2 marks)
- 1.4 A scientist thinks there may be a relationship between the pitch of a songbird's song and its body size. She records the body size and song pitch of songbird B and 50 individuals of the same species. What statistical test could she carry out on her results to determine whether there is a relationship between song pitch and body size? Explain your answer.
- (2 marks)

- 2 The RuBisCo gene is found in all plants. When a new species of plant is being classified, this gene is often compared with the gene in other species to determine evolutionary relatedness.
- 2.1 Explain why the RuBisCo gene is useful for determining relationships between plant species.
- (1 mark)
- 2.2 Evolutionary relationships could also be determined by comparing the RuBisCo protein itself. Describe **one** way in which proteins from two different organisms could be used to determine evolutionary relationships.
- (2 marks)
- 3 A team of students have investigated plant biodiversity on two farms. To do so, they calculated an index of diversity. Species richness can also be used to measure biodiversity.
- 3.1 Explain why an index of diversity is a more accurate way of measuring biodiversity than species richness.

Table 1

(2 marks)

Table 1 shows the number of individuals of different plant species found in a single hedgerow on each farm.

| Plant Species | Farm A | Farm B |
|---------------|--------|--------|
| A | 3 | 12 |
| B | 6 | 2 |
| C | 9 | 4 |
| D | 7 | 6 |
| E | 11 | 3 |
| F | 11 | 0 |

- 3.2 Calculate the index of diversity for the hedgerow on each farm using the equation provided below.

$$d = \frac{N(N - 1)}{\sum n(n-1)} \quad \text{where, } N = \text{total number of all organisms}$$

and $n = \text{total number of organisms in one species.}$

(4 marks)

- 3.3 One of the farms grows organic crops and does not use chemical herbicides. Which farm is this most likely to be? Explain your answer.
- (2 marks)
- 3.4 Many organic farms use biological pesticides. These include introducing organisms that prey on the pests that eat crops. The students behind the first study want to investigate the impact of biological pesticides on insect species diversity in farm hedgerows.
- Suggest a control the students might use in their investigation.
- (1 mark)
- The government offers grants to farmers to maintain their hedgerows.
- 3.5 Suggest **one** advantage to farmers of removing hedgerows from their land.
- (1 mark)
- 3.6 Suggest what impact hedgerow removal could have on insect biodiversity on the farm. Explain your answer.
- (3 marks)

Topic 5 A: Photosynthesis and Respiration

1. Photosynthesis, Respiration and Energy

The ability to store, transfer and release energy is really important for plants and animals. That's where photosynthesis and respiration come in.

Why is energy important?

Plant and animal cells need energy for biological processes to occur.

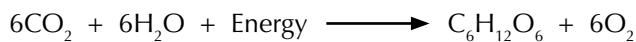
Examples

- Plants need energy for things like photosynthesis, active transport (e.g. to take in minerals via their roots), DNA replication, cell division and protein synthesis.
- Animals need energy for things like muscle contraction, maintenance of body temperature, active transport, DNA replication, cell division and protein synthesis.

Tip: Without energy, the biological processes described would stop and the plant or animal would die.

Photosynthesis and energy

Photosynthesis is the process where energy from light is used to make glucose from water (H_2O) and carbon dioxide (CO_2). The light energy is converted to chemical energy in the form of glucose — $C_6H_{12}O_6$. The overall equation is:



Energy is stored in the glucose until the plants (or other photosynthesising organisms, e.g. algae) release it by respiration. Animals obtain glucose by eating plants (or by eating other animals, which have eaten plants), then respire the glucose to release energy.

Photosynthesis is an example of a **metabolic pathway** — the process occurs in a series of small reactions controlled by enzymes.

Tip: Any organism that carries out photosynthesis is known as a 'photoautotroph' (an organism that can make its own food using light energy). The process of photosynthesis is the same in all photoautotrophs, suggesting that they all evolved from a common ancestor.

Respiration and energy

Plant and animal cells release energy from glucose — this process is called respiration. This energy is used to power all the biological processes in a cell. There are two types of respiration:

- Aerobic respiration** — respiration using oxygen.
- Anaerobic respiration** — respiration without oxygen.

Aerobic respiration produces carbon dioxide and water, and releases energy. The overall equation is:



Anaerobic respiration in plants and yeast produces ethanol and carbon dioxide and releases energy. In humans, anaerobic respiration produces lactate and releases energy. Aerobic and anaerobic respiration are both examples of metabolic pathways.

Tip: Energy is never created or destroyed. It's always converted from one form to another. For example, in photosynthesis light energy is converted to chemical energy (glucose). This energy is then used to fuel biological processes.

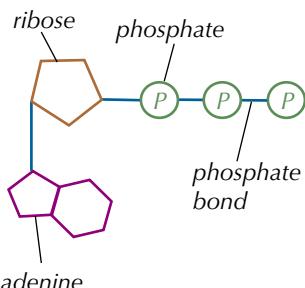
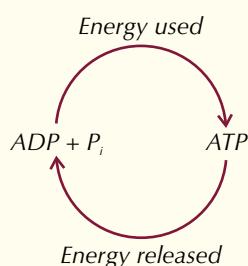


Figure 1: The structure of adenosine triphosphate (ATP). It consists of adenine, ribose and three phosphate groups.

Tip: Adenosine diphosphate has two phosphate groups. Adenosine triphosphate has three phosphate groups.

Tip: In a cell there's a constant cycle between ADP and P_i , and ATP. This allows energy to be stored and released as it's needed.



Tip: It's important to remember that ATP isn't energy — it's a store of energy. Energy is used to make ATP, then it's released when ATP is hydrolysed to ADP and P_i .

ATP

As you learnt in Topic 1, ATP (adenosine triphosphate) is the immediate source of energy in a cell.

A cell can't get its energy directly from glucose. So, in respiration, the energy released from glucose is used to make ATP. ATP is made from the nucleotide base adenine, combined with a ribose sugar and three phosphate groups (see Figure 1). It carries energy around the cell to where it's needed.

ATP is synthesised via a condensation reaction between ADP (adenosine diphosphate) and inorganic phosphate (P_i) using energy from an energy-releasing reaction, e.g. the breakdown of glucose in respiration. The energy is stored as chemical energy in the phosphate bond (see Figure 2). The enzyme **ATP synthase** catalyses this reaction.

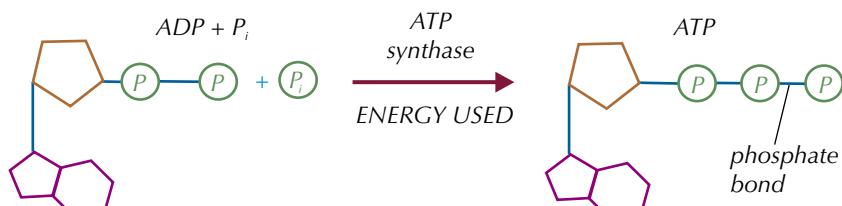


Figure 2: The synthesis of ATP.

This process is known as **phosphorylation** — adding phosphate to a molecule. ADP is phosphorylated to ATP.

ATP then diffuses to the part of the cell that needs energy. Here, it's broken down back into ADP and inorganic phosphate (P_i). Chemical energy is released from the phosphate bond and used by the cell. **ATP hydrolase** catalyses this reaction.

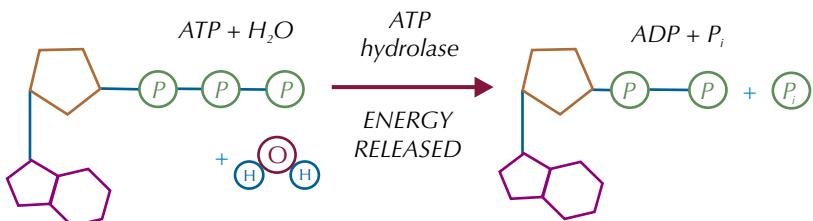


Figure 3: The breakdown of ATP.

This process is known as **hydrolysis**. It's the splitting (lysis) of a molecule using water (hydro). The ADP and inorganic phosphate are recycled and the process starts again.

ATP's properties

ATP has specific properties that make it a good energy source.

- ATP stores or releases only a small, manageable amount of energy at a time, so no energy is wasted as heat.
- It's a small, soluble molecule so it can be easily transported around the cell.
- It's easily broken down, so energy can be easily released instantaneously.
- It can be quickly remade.
- It can make other molecules more reactive by transferring one of its phosphate groups to them (phosphorylation).
- ATP can't pass out of the cell, so the cell always has an immediate supply of energy.

The compensation point

Plants carry out both photosynthesis and respiration. Both processes can occur at the same time and at different rates. The rate at which photosynthesis takes place is partly dependent on the light intensity of the environment that the plant is in (see page 272).

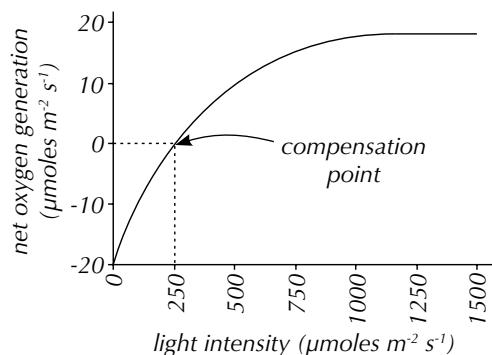
There's a particular level of light intensity at which the rate of photosynthesis exactly matches the rate of respiration. This is called the **compensation point** for light intensity.

One way to work out the compensation point for a plant is to measure the rate at which oxygen is produced and used by a plant at different light intensities. Because photosynthesis produces oxygen and respiration uses it, in this case, the compensation point is the light intensity at which oxygen is being used as quickly as it is produced (see the example below). The rate of CO_2 production and use could also be measured — photosynthesis uses CO_2 and respiration produces it.

Tip: The products of photosynthesis (e.g. O_2) can be used as reactants in respiration and vice versa. Reactants can also come from elsewhere (e.g. O_2 can come from air).

Example — Maths Skills

The graph below shows the net oxygen generation by a plant grown in a controlled environment under different light intensities. When the rate of oxygen production equals the rate of oxygen usage, oxygen generation is zero. This is the compensation point.



In this example, the compensation point occurs at a light intensity of **250 $\mu\text{moles m}^{-2} \text{s}^{-1}$** .

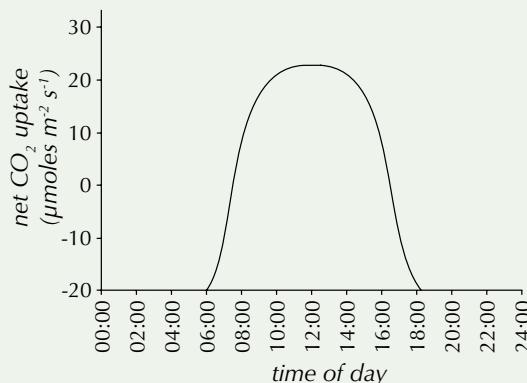
Tip: The compensation point is different for different species of plants.

Exam Tip

$\mu\text{moles m}^{-2} \text{s}^{-1}$ (micromoles per metre squared per second) is a unit that can be used when measuring light intensity. Don't panic if you see unfamiliar units like this in the exam — just focus on what the axis is showing you, e.g. here it's light intensity.

Practice Question — Application

- Q1 The graph on the right shows the CO_2 uptake of a plant over the course of a day in early spring.
- Give the times when compensation points occur.
 - Suggest and explain why the compensation points occur at these particular times.



Exam Tip

Graphs showing the compensation point won't always show oxygen generation. If you haven't seen the factors used on the scales of the graph in the exam before, don't panic. Just remember that the compensation point is the point at which photosynthesis and respiration are occurring at the same rate and apply your knowledge to work it out from the graph you've been given.

Learning Objective:

- Know the light-dependent reaction in such detail as to show that:
 - chlorophyll absorbs light, leading to photoionisation of chlorophyll,
 - some of the energy from electrons released during photoionisation is conserved in the production of ATP and reduced NADP,
 - the production of ATP involves electron transfer associated with the transfer of electrons down the electron transfer chain and passage of protons across chloroplast membranes, and is catalysed by ATP synthase embedded in these membranes (chemiosmotic theory),
 - photolysis of water produces protons, electrons and oxygen.

Specification Reference 3.5.1

Tip: One way to remember electron and hydrogen movement is OILRIG. **Oxidation Is Loss, Reduction Is Gain.**

Tip: When hydrogen is transferred between molecules, electrons are transferred too.

2. Photosynthesis and the Light-dependent Reaction

In photosynthesis, light energy is used to make glucose. It involves a series of reactions, but before we get stuck into it you need to know a bit of background information...

Chloroplasts

Photosynthesis takes place in the chloroplasts of plant cells. Chloroplasts are small, flattened organelles surrounded by a double membrane (see Figure 1).

Thylakoids (fluid-filled sacs) are stacked up in the chloroplast into structures called **grana** (singular = granum). The grana are linked together by bits of thylakoid membrane called **lamellae** (singular = lamella).

Chloroplasts contain **photosynthetic pigments** (e.g. chlorophyll a, chlorophyll b and carotene). These are coloured substances that absorb the light energy needed for photosynthesis. The pigments are found in the thylakoid membranes

— they're attached to proteins. The protein and pigment is called a **photosystem**. There are two photosystems used by plants to capture light energy. Photosystem I (or PSI) absorbs light best at a wavelength of 700 nm and photosystem II (PSII) absorbs light best at 680 nm.

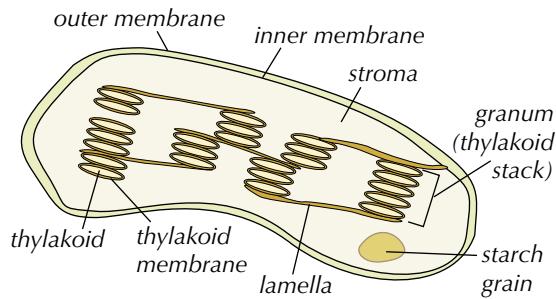


Figure 1: The structure of a chloroplast.

Contained within the inner membrane of the chloroplast and surrounding the thylakoids is a gel-like substance called the **stoma**

— see Figure 1. It contains enzymes, sugars and organic acids. Carbohydrates that are produced by photosynthesis but not used straight away are stored as starch grains in the stroma.

Redox reactions

Redox reactions are reactions that involve **oxidation** and **reduction**. They occur in photosynthesis (and in respiration) so it's really important that you get your head round them:

- If something is reduced it has gained electrons (e^-), and may have gained hydrogen or lost oxygen.
- If something is oxidised it has lost electrons, and may have lost hydrogen or gained oxygen.
- Oxidation of one molecule always involves reduction of another molecule.

Coenzymes

A coenzyme is a molecule that aids the function of an enzyme. They work by transferring a chemical group from one molecule to another. A coenzyme used in photosynthesis is **NADP**. NADP transfers hydrogen from one molecule to another — this means it can reduce (give hydrogen to) or oxidise (take hydrogen from) a molecule.

The stages of photosynthesis

There are actually two stages that make up photosynthesis — the light-dependent reaction and the light-independent reaction. The next few pages are all about the light-dependent reaction, but before we get into all that you need to know how the two stages link together.

1. The light-dependent reaction

As the name suggests, this reaction needs light energy — see Figure 3. It takes place in the thylakoid membranes of the chloroplasts. Here, light energy is absorbed by chlorophyll (and other photosynthetic pigments) in the photosystems. The light energy excites the electrons in the chlorophyll, giving them more energy, which eventually causes them to be released from the chlorophyll molecule. This process is called **photoionisation**. The chlorophyll molecule is now a positively charged ion.

Some of the energy from the released electrons is used to add a phosphate group to ADP to form ATP, and some is used to reduce NADP to form reduced NADP. ATP transfers energy and reduced NADP transfers hydrogen to the light-independent reaction. During the process, H_2O is oxidised to O_2 .

2. The light-independent reaction (the Calvin cycle)

As the name suggests, this reaction doesn't use light energy directly. (But it does rely on the products of the light-dependent reaction.) It takes place in the stroma of the chloroplast — see Figure 3. Here, the ATP and reduced NADP from the light-dependent reaction supply the energy and hydrogen to make glucose from CO_2 .

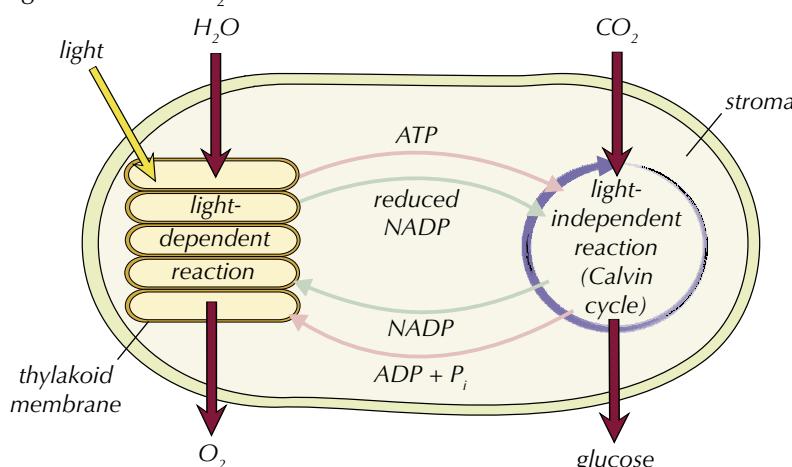


Figure 3: How the light-dependent and light-independent reactions link together in a chloroplast.

The light-dependent reaction

In the light-dependent reaction, the energy resulting from the photoionisation of chlorophyll is used for three things:

1. Making ATP from ADP and inorganic phosphate. This is called **photophosphorylation** — it's the process of adding phosphate to a molecule using light.
2. Making reduced NADP from NADP.
3. Splitting water into protons (H^+ ions), electrons and oxygen. This is called **photolysis** — it's the splitting (lysis) of a molecule using light (photo) energy.

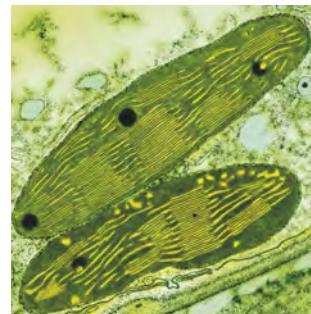


Figure 2: A cross-sectional image of two chloroplasts.

Tip: Reduced NADP is also written as NADPH — it's NADP that's gained a hydrogen. Remember OILRIG (see previous page) — reduction is gain.

Tip: See pages 269-271 for loads more information on the Calvin cycle.

Tip: The light-independent reaction can take place in the dark. However, it needs the products of the light-dependent reaction, (ATP and reduced NADP) so in reality it only continues for a little while after it gets dark.

The light-dependent reaction actually includes two types of photophosphorylation — non-cyclic and cyclic. Each of these processes has different products and is explained on the next couple of pages.

Tip: To remind yourself what photosystems are, take a look back at page 264.

Tip: Not all of the electron carriers are shown in these diagrams.

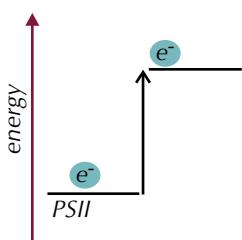


Figure 4: Light energy excites electrons in PSII, moving them to a higher energy level.

Tip: So the O₂ in photosynthesis comes from water and is made in the light-dependent reaction. It diffuses out of the chloroplast and eventually into the atmosphere for us to breathe. Good old plants.

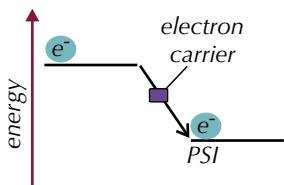


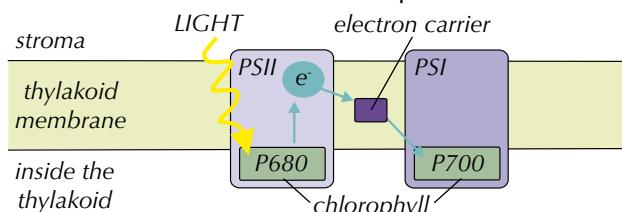
Figure 5: The excited electrons lose energy as they pass down the electron transport chain.

Non-cyclic photophosphorylation

Non-cyclic photophosphorylation produces ATP, reduced NADP and oxygen (O₂). To understand the process you need to know that the photosystems (in the thylakoid membranes) are linked by **electron carriers**. Electron carriers are proteins that transfer electrons. The photosystems and electron carriers form an **electron transport chain** — a chain of proteins through which excited electrons flow. There are several processes going on all at once in non-cyclic photophosphorylation — they're shown separately in the diagrams below.

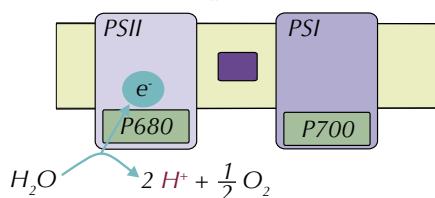
1. Light energy excites electrons in chlorophyll

Light energy is absorbed by PSII. The light energy excites electrons in chlorophyll. The electrons move to a higher energy level (i.e. they have more energy — see Figure 4). These high-energy electrons are released from the chlorophyll and move down the electron transport chain to PSI.



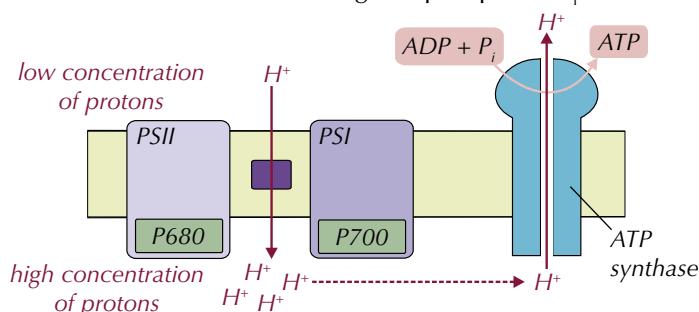
2. Photolysis of water produces protons, electrons and oxygen

As the excited electrons from chlorophyll leave PSII to move down the electron transport chain, they must be replaced. Light energy splits water into protons (H⁺ ions), electrons and oxygen — this is photolysis. The reaction is: H₂O → 2H⁺ + ½O₂



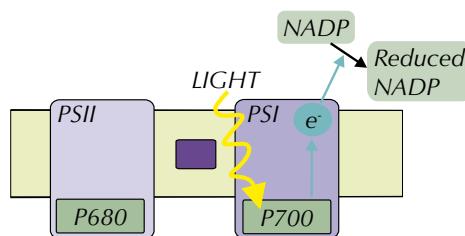
3. Energy from the excited electrons makes ATP

The excited electrons lose energy as they move down the electron transport chain (see Figure 5). This energy is used to transport protons (H⁺ ions) into the thylakoid so that the thylakoid has a higher concentration of protons than the stroma. This forms a proton gradient across the thylakoid membrane. Protons move down their concentration gradient, into the stroma, via the enzyme ATP synthase, which is embedded in the thylakoid membrane. The energy from this movement combines ADP and inorganic phosphate (P_i) to form ATP.

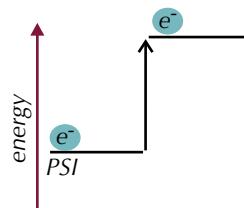


4. Energy from the excited electrons generates reduced NADP

Light energy is absorbed by PSII, which excites the electrons again to an even higher energy level. Finally, the electrons are transferred to NADP, along with a proton (H^+ ion) from the stroma, to form reduced NADP.



Tip: Remember a 'proton' is just another word for a hydrogen ion (H^+).

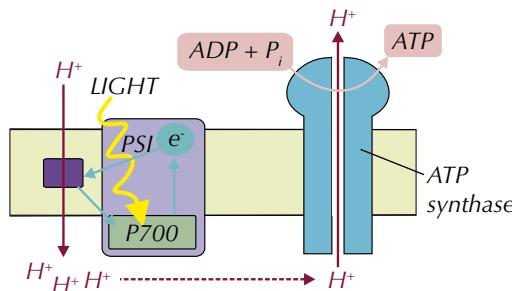


Chemiosmotic theory

The process of electrons flowing down the electron transport chain and creating a proton gradient across the membrane to drive ATP synthesis is called chemiosmosis. It's described by the chemiosmotic theory.

Cyclic photophosphorylation

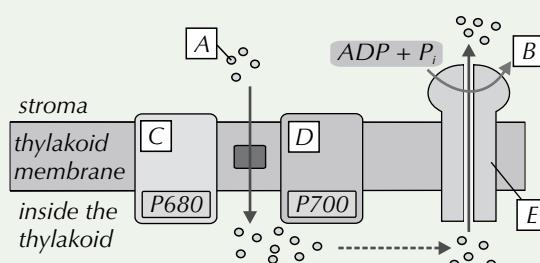
Cyclic photophosphorylation produces ATP and only uses PSI. It's called 'cyclic' because the electrons from the chlorophyll molecule aren't passed onto NADP, but are passed back to PSI via electron carriers. This means the electrons are recycled and can repeatedly flow through PSI. This process doesn't produce any reduced NADP or oxygen — it only produces small amounts of ATP.



Tip: The ATP and reduced NADP made here in the light-dependent reaction are really important for use later on in the light-independent reaction (see page 269).

Practice Questions — Application

- Q1 This diagram on the right shows a process in the light-dependent reaction.

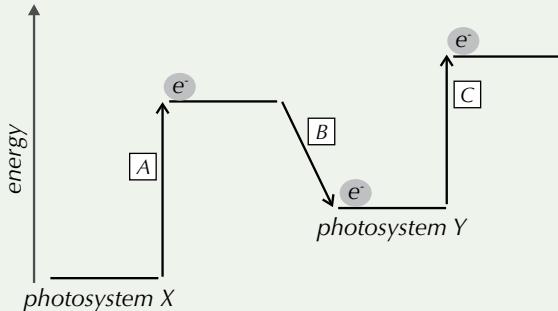


- The object labelled A in the diagram is transported across the thylakoid membrane, so that its concentration is higher in the thylakoid than in the stroma.
 - What is the name of object A?
 - Explain why it is important that the concentration of object A is higher inside the thylakoid than in the stroma.

Tip: Tempting as it is, you need to be able to answer this question without looking back at the last couple of pages.

Tip: Q1 continues on the next page.

- b) What is the name of structure C?
 c) Which structure, C or D, is involved in cyclic photophosphorylation?
 d) What does cyclic photophosphorylation produce?
- Q2 The diagram below shows the energy levels of electrons at different stages of the light-dependent reaction of photosynthesis.



- a) What are the correct names of photosystems X and Y?
 b) Explain what is happening at stage A on the diagram.
 c) Electrons lose energy at stage B in the diagram.
 What is this energy used for?
 d) At point C in the diagram, electrons reach their highest energy level. What happens to the electrons after this point in non-cyclic photophosphorylation?

Practice Questions — Fact Recall

- Q1 a) What are photosynthetic pigments?
 b) Give one example of a photosynthetic pigment.
- Q2 NADP is a coenzyme used in photosynthesis.
 What chemical group does it transfer between molecules?
- Q3 Where in the chloroplast does the light-dependent reaction take place?
- Q4 Describe what happens during the photoionisation of chlorophyll.
- Q5 Which products of the light-dependent reaction are needed in the light-independent reaction?
- Q6 What is photophosphorylation?
- Q7 What is the electron transport chain?
- Q8 a) Name the products of the photolysis of water.
 b) What is the purpose of photolysis in the light-dependent reaction?
- Q9 Excited electrons lose energy as they move down the electron transport chain. Explain how this leads to ATP synthesis.
- Q10 Name the products of:
 a) non-cyclic photophosphorylation,
 b) cyclic photophosphorylation.

Tip: Make sure you get your head round what happens in cyclic and non-cyclic phosphorylation (see pages 266-267) — don't get them mixed up.

3. Photosynthesis and the Light-independent Reaction

The light-independent reaction is the second (and final, phew) stage of photosynthesis. It uses the products of the light-dependent reaction (ATP and reduced NADP) to make organic substances for the plant.

The Calvin cycle

The light-independent reaction is also called the Calvin cycle. It takes place in the stroma of the chloroplasts. It makes a molecule called **triose phosphate** from carbon dioxide (CO_2) and **ribulose bisphosphate** (a 5-carbon compound). Triose phosphate can be used to make glucose and other useful organic substances. There are a few steps in the cycle, and it needs ATP and H^+ ions to keep it going. The reactions are linked in a cycle (see Figure 1), which means the starting compound, ribulose bisphosphate, is regenerated.

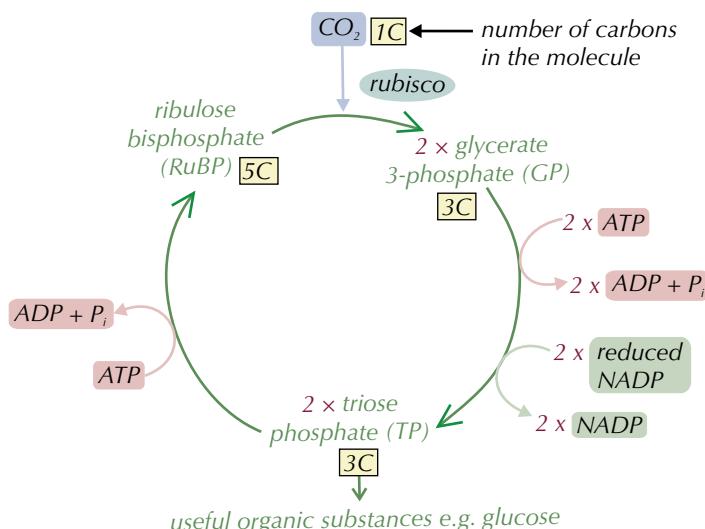


Figure 1: One turn of the Calvin cycle.

Here's what happens at each stage in the cycle:

1. Formation of glycerate 3-phosphate

CO_2 enters the leaf through the stomata and diffuses into the stroma of the chloroplast. Here, it's combined with ribulose bisphosphate (RuBP). This reaction is catalysed by the enzyme **rubisco**. This gives an unstable 6-carbon compound, which quickly breaks down into two molecules of a 3-carbon compound called **glycerate 3-phosphate** (GP).



2. Formation of triose phosphate

The hydrolysis of ATP (from the light-dependent reaction) provides energy to **reduce** the 3-carbon compound, GP, to a different 3-carbon compound called triose phosphate (TP). This reaction also requires H^+ ions, which come from reduced NADP (also from the light-dependent reaction). Reduced NADP is recycled to NADP. This is shown in the diagram at the top of the next page. Some triose phosphate is then converted into useful organic compounds (e.g. glucose) and some continues in the Calvin cycle to regenerate RuBP.

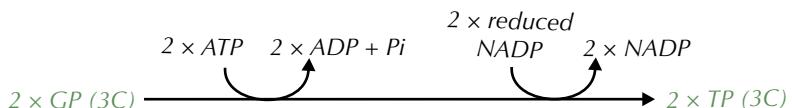
Learning Objectives:

- Know that the light-independent reaction uses reduced NADP from the light-dependent reaction to form a simple sugar, and that the hydrolysis of ATP, also from the light-dependent reaction, provides the additional energy for this reaction.
- Know the light-independent reaction in such detail to show that:
 - carbon dioxide reacts with ribulose bisphosphate (RuBP) to form two molecules of glycerate 3-phosphate (GP) and that this reaction is catalysed by the enzyme rubisco,
- ATP and reduced NADP from the light-dependent reaction are used to reduce GP to triose phosphate,
- some of the triose phosphate is used to regenerate RuBP in the Calvin cycle,
- some of the triose phosphate is converted to useful organic substances.

Specification Reference 3.5.1

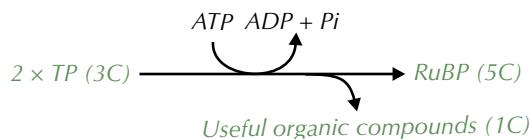
Exam Tip

Don't panic if you get a diagram of the Calvin cycle in the exam that doesn't look exactly the same as the one above — as long as you remember the key points shown here then you'll be fine.



3. Regeneration of ribulose bisphosphate

Five out of every six molecules of TP produced in the cycle aren't used to make useful organic compounds, but to regenerate RuBP. Regenerating RuBP uses the rest of the ATP produced by the light-dependent reaction.



Hexose sugars

Hexose sugars are simple 6-carbon sugars, e.g. glucose (see Figure 2). One hexose sugar is made by joining two molecules of triose phosphate (TP) together. Hexose sugars can be used to make larger carbohydrates (see next page).

The Calvin cycle needs to turn six times to make one hexose sugar. The reason for this is that three turns of the cycle produce six molecules of triose phosphate (because two molecules of TP are made for every one CO_2 molecule used). Five out of six of these TP molecules are used to regenerate ribulose bisphosphate (RuBP). This means that for three turns of the cycle, only one TP is produced that's used to make a hexose sugar.

A hexose sugar has six carbons though, so two TP molecules are needed to form one hexose sugar. This means the cycle must turn six times to produce two molecules of TP that can be used to make one hexose sugar — see Figure 3. Six turns of the cycle need 18 ATP and 12 reduced NADP from the light-dependent reaction.

This might seem a bit inefficient, but it keeps the cycle going and makes sure there's always enough RuBP ready to combine with CO_2 taken in from the atmosphere.

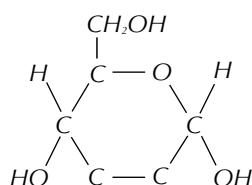


Figure 2: The structure of glucose, a hexose sugar.

Exam Tip

If you're asked in the exam to work out how many turns of the Calvin cycle are needed to produce a certain number of hexose sugars you need to remember that five out of every six TP molecules are used to regenerate RuBP.

Tip: Six turns of the Calvin cycle produce 12 GP molecules because one turn produces 2 GP, so $6 \times 2 = 12$ GP.

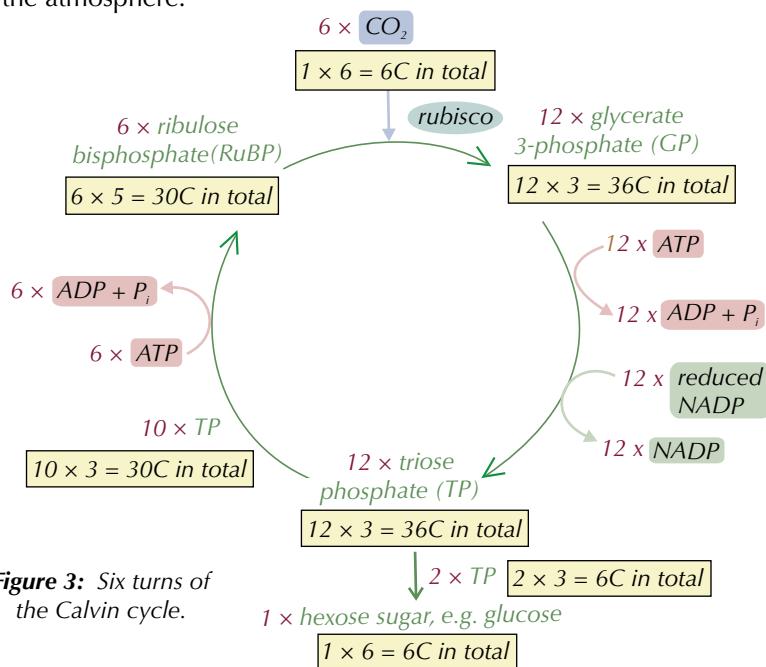


Figure 3: Six turns of the Calvin cycle.

Carbohydrates, lipids and proteins

The Calvin cycle is the starting point for making all the organic substances a plant needs. Triose phosphate (TP) and glycerate 3-phosphate (GP) molecules are used to make carbohydrates, lipids and amino acids:

- **Carbohydrates** — hexose sugars are made from two triose phosphate molecules (see the previous page) and larger carbohydrates (e.g. sucrose, starch, cellulose — see Figure 4) are made by joining hexose sugars together in different ways.
- **Lipids** — these are made using glycerol, which is synthesised from triose phosphate, and fatty acids, which are synthesised from glycerate 3-phosphate.
- **Amino acids** — some amino acids are made from glycerate 3-phosphate.

Tip: The Calvin cycle is also called carbon fixation, because carbon from CO_2 is ‘fixed’ into an organic molecule.



Figure 4: Cellulose strands in a plant cell wall made from hexose sugars.

Practice Questions — Application

- Q1 Rubisco is an enzyme that catalyses the first reaction in the Calvin cycle. Some scientists are trying to genetically modify rubisco to try to increase the speed at which it works. They believe if they can make rubisco work faster then plants will be able to produce organic substances, such as glucose, more quickly. Use your knowledge of photosynthesis to explain how increasing the speed of rubisco could increase the speed of glucose production.
- Q2 Phosphoribulokinase is an enzyme involved in the regeneration of ribulose bisphosphate. If this enzyme stopped working properly, suggest what effect it would have on the light-independent reaction of photosynthesis in a plant. Explain your answer.

Tip: Rubisco is one of the slowest-working enzymes in the natural world.

Practice Questions — Fact Recall

- Q1 a) What is the name of the 5-carbon compound that combines with carbon dioxide to form an unstable 6-carbon compound in the first reaction of the Calvin cycle?
- b) The 6-carbon compound produced only exists fleetingly before it breaks down into two molecules of a 3-carbon compound. What is the name of this 3-carbon compound?
- Q2 a) Write out a word equation to show the formation of two molecules of triose phosphate.
- b) Is this reaction an oxidation or reduction reaction?
- Q3 Describe the role of ATP in the Calvin cycle.
- Q4 If six molecules of triose phosphate (TP) are produced by the Calvin cycle, how many of these will be used to regenerate ribulose bisphosphate?
- Q5 To make one hexose sugar:
- How many turns of the Calvin cycle are needed?
 - How many molecules of ATP are needed?
 - How many molecules of reduced NADP are needed?
- Q6 Describe how the products of the Calvin cycle are used to make:
- large carbohydrates
 - lipids

Tip: The Calvin cycle can be summarised as follows:

Inputs

CO_2
ATP
Reduced NADP



Outputs

Organic substances
 RuBP

Learning Objectives:

- Be able to identify environmental factors that limit the rate of photosynthesis.
- Be able to evaluate data relating to common agricultural practices used to overcome the effect of these limiting factors.

Specification Reference 3.5.1

Tip: Green light is reflected, which is why plants look green.

Tip: When an enzyme becomes denatured, the bonds holding its tertiary structure together break. It loses its 3D shape so the active site won't fit the substrate. The enzyme can no longer function as a catalyst.

Tip: Remember: stomata are pores in the epidermis of a plant that allow gas exchange.

Tip: There's less oxygen in waterlogged soil, so roots are unable to respire aerobically. This means there's less ATP available for the active transport of minerals into roots.

4. Limiting Factors in Photosynthesis

Plants have optimum conditions for photosynthesis. If you're a budding gardener then these pages are for you...

Optimum conditions for photosynthesis

The ideal conditions for photosynthesis vary from one plant species to another, but the conditions below would be ideal for most plant species in temperate climates like the UK.

1. High light intensity of a certain wavelength

Light is needed to provide the energy for the light-dependent reaction — the higher the intensity of the light, the more energy it provides.

Only certain wavelengths of light are used for photosynthesis.

The photosynthetic pigments chlorophyll a, chlorophyll b and carotene only absorb the red and blue light in sunlight (see Figure 1).

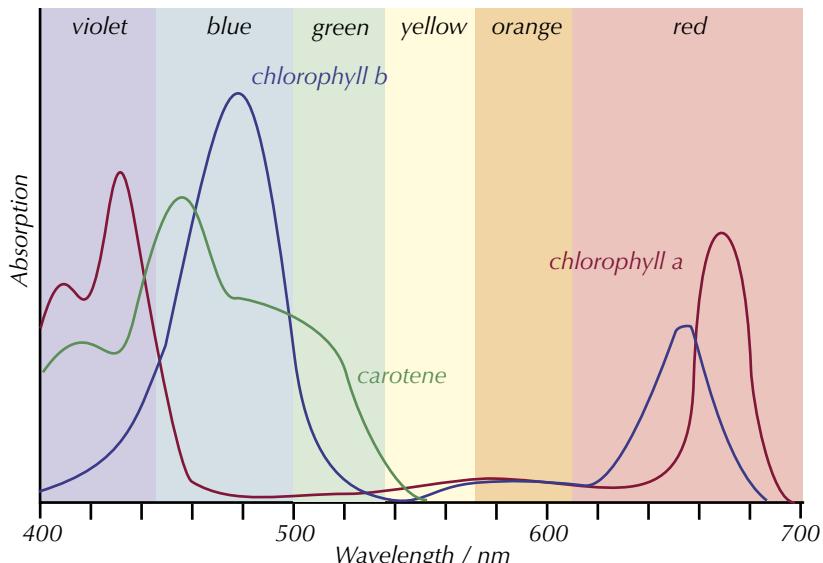


Figure 1: The wavelengths of light absorbed by chlorophylls a and b, and carotene.

2. Temperature around 25 °C

Photosynthesis involves enzymes (e.g. ATP synthase, rubisco). If the temperature falls below 10 °C the enzymes become inactive, but if the temperature is more than 45 °C they may start to **denature**. Also, at high temperatures stomata close to avoid losing too much water. This causes photosynthesis to slow down because less carbon dioxide enters the leaf when the stomata are closed.

3. Carbon dioxide at 0.4%

Carbon dioxide makes up 0.04% of the gases in the atmosphere. Increasing this to 0.4% gives a higher rate of photosynthesis, but any higher and the stomata start to close.

4. Water

Plants also need a constant supply of water — too little and photosynthesis has to stop but too much and the soil becomes waterlogged (reducing the uptake of minerals such as magnesium, which is needed to make chlorophyll a).

Limiting factors of photosynthesis

Light, temperature and carbon dioxide can all limit photosynthesis. All three of these things need to be at the right level to allow a plant to photosynthesise as quickly as possible. If any one of these factors is too low or too high, it will limit photosynthesis (slow it down). Even if the other two factors are at the perfect level, it won't make any difference to the speed of photosynthesis as long as that factor is at the wrong level.

Tip: A limiting factor is a variable that can slow down the rate of a reaction.

Examples

- On a warm, sunny, windless day, it's usually carbon dioxide that's the limiting factor.
- At night it's the light intensity that's the limiting factor.

However, any of these factors could become the limiting factor, depending on the environmental conditions. The graphs below show the effect of each limiting factor on the rate of photosynthesis:

Examples

Light intensity

Between points A and B, the rate of photosynthesis is limited by the light intensity. So as the light intensity increases, so can the rate of photosynthesis. Point B is the **saturation point** — increasing light intensity after this point makes no difference, because something else has become the limiting factor. The graph now levels off.

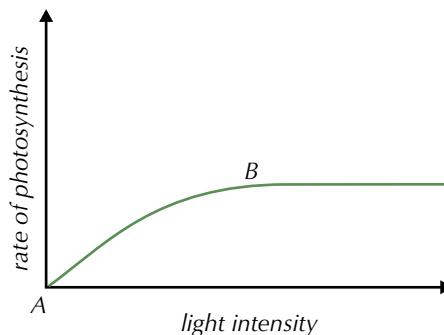
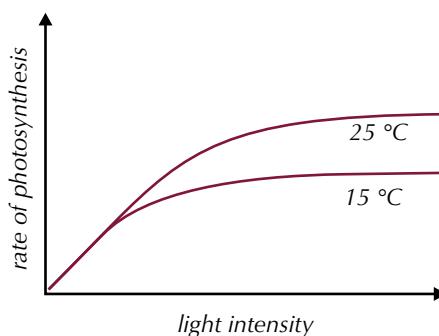


Figure 2: As night falls, light intensity begins to limit the rate of photosynthesis.

Temperature

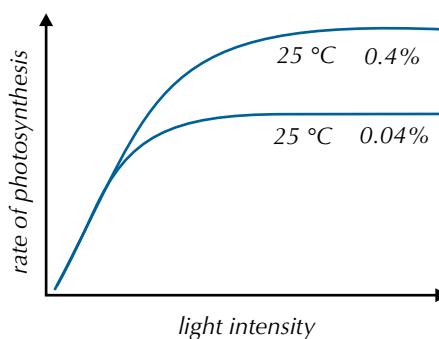
Both these graphs level off when light intensity is no longer the limiting factor. The graph at 25 °C levels off at a higher point than the one at 15 °C, showing that temperature must have been a limiting factor at 15 °C.



Tip: The saturation point is where a factor is no longer limiting the reaction — something else has become the limiting factor.

Carbon dioxide concentration

Both these graphs level off when light intensity is no longer the limiting factor. The graph at 0.4% carbon dioxide (CO₂) levels off at a higher point than the one at 0.04%, so carbon dioxide concentration must have been a limiting factor at 0.04% carbon dioxide. The limiting factor here isn't temperature because it's the same for both graphs (25 °C).



Tip: As each of the graphs level off, it doesn't mean that photosynthesis has stopped — it means that the rate of photosynthesis is not increasing anymore.

Tip: A greenhouse is the same thing as a glasshouse.

Tip: Similar techniques can also be used in polytunnels (tunnels made of polythene, under which plants can be grown).



Figure 3: Lamps in greenhouses provide light at night.

Tip: Remember the wavelength of light is also important. Agricultural growers will often use red or blue lights to maximise photosynthesis. If they used green light it would be reflected by the plants — see page 272.

Tip: In this study, the negative control was growing plants in a greenhouse where CO_2 wasn't added. Using this control would have made sure that no other factors, apart from the level of CO_2 , were affecting the results. For more information on controls see page 2.

Increasing plant growth

Agricultural growers (e.g. farmers) know the factors that limit photosynthesis and therefore limit plant growth. This means they try to create an environment where plants get the right amount of everything that they need, which increases growth and so increases yield. Growers create optimum conditions in **glasshouses**, in the following ways:

| Limiting Factor | Management in Glasshouse |
|------------------------------|--|
| Carbon dioxide concentration | Carbon dioxide is added to the air, e.g. by burning a small amount of propane in a carbon dioxide generator. |
| Light | Light can get in through the glass. Lamps provide light at night time. |
| Temperature | Glasshouses trap heat energy from sunlight, which warms the air. Heaters and cooling systems can also be used to keep a constant optimum temperature, and air circulation systems make sure the temperature is even throughout the glasshouse. |

Interpreting data on limiting factors

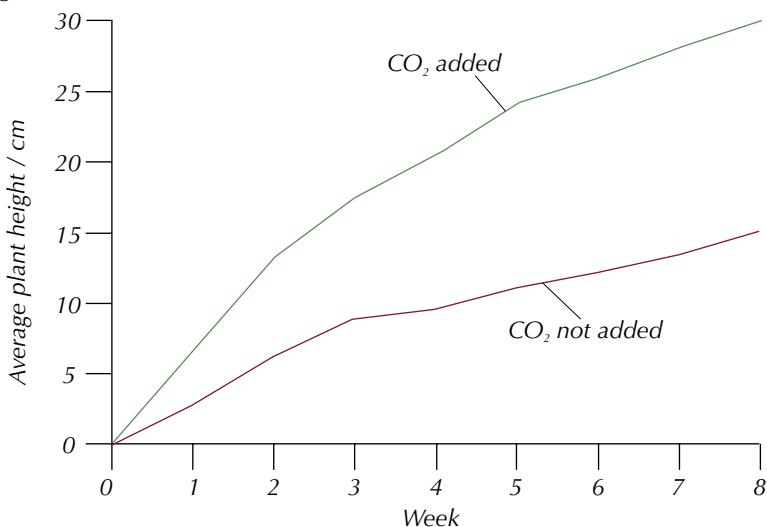
You need to be able to interpret data on limiting factors.

Here are some examples of the kind of data you might get in the exam:

Examples

Carbon dioxide

The graph below shows the effect on plant growth of adding carbon dioxide to a greenhouse.

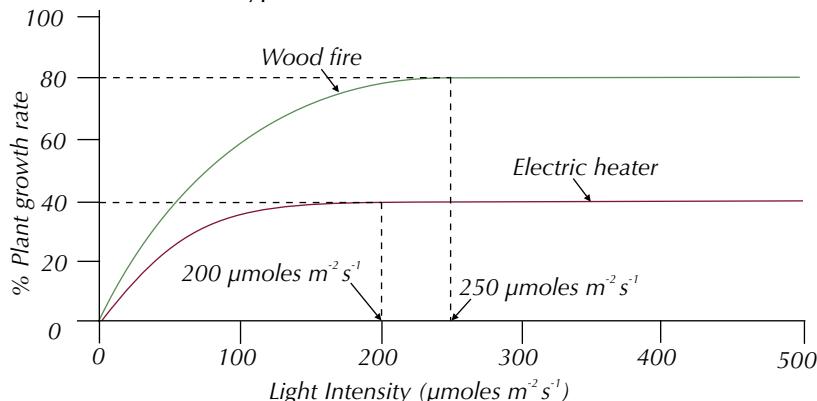


In the greenhouse with added carbon dioxide plant growth was faster (the line is steeper) and on average the plants were larger after 8 weeks than they were in the control greenhouse (30 cm compared to only 15 cm in the greenhouse where no carbon dioxide was added).

This is because the plants use carbon dioxide to produce glucose by photosynthesis. The more carbon dioxide they have, the more glucose they can produce, meaning they can respire more and so have more ATP for DNA replication, cell division and protein synthesis, i.e. growth.

Light intensity

The graph below shows the effect of light intensity on plant growth, and the effect of two different types of heater:

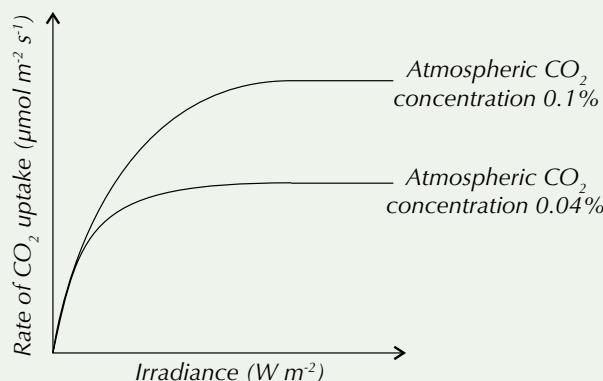


- At the start of the graph, the greater the light intensity the greater the plant growth.
- At 200 $\mu\text{moles m}^{-2} \text{s}^{-1}$ of light the bottom graph flattens out, showing that carbon dioxide concentration or temperature is limiting growth in these plants.
- At 250 $\mu\text{moles m}^{-2} \text{s}^{-1}$ of light the top graph flattens out. The difference between the two graphs could be because the wood fire increases the temperature more than the electric heater or because it's increasing the concentration of carbon dioxide in the air (an electric heater doesn't release carbon dioxide).

Tip: If you were conducting this experiment you would have to measure the temperature and the carbon dioxide concentration in each situation to be able to decide which was actually the factor limiting photosynthesis.

Practice Question — Application

- Q1 An agricultural scientist is investigating the effect of irradiance (the amount of light energy hitting a surface) and increasing atmospheric CO_2 concentration on the rate of CO_2 uptake by a tomato crop. The results are shown in the graph below. The plants were grown in laboratory conditions, in which temperature was kept constant.



- Explain why the rate of CO_2 uptake initially increases with increasing irradiance.
- Explain why both lines on the graph eventually level off.
- The scientist concluded that using a paraffin heater to increase the CO_2 in a glasshouse would improve the tomato yield. Evaluate how far the data supports this conclusion.

Learning Objectives:

- Be able to use chromatography to investigate the pigments isolated from leaves of different plants, e.g. leaves from shade-tolerant and shade-intolerant plants or leaves of different colours (Required Practical 7).
- Be able to carry out an investigation into the effect of a named factor on the rate of dehydrogenase activity in extracts of chloroplasts (Required Practical 8).

Specification Reference 3.5.1

Tip: The pattern of spots you end up with is called a chromatogram.

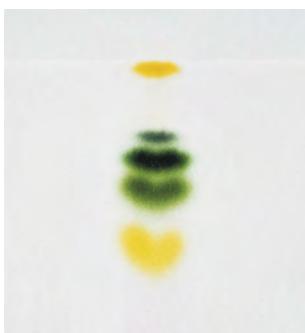


Figure 2: Plant pigments that have been separated by thin-layer chromatography.

5. Photosynthesis Experiments

You need to know how to carry out two types of experiment related to different aspects of photosynthesis for your exams.

Chromatography

Chromatography is used to separate stuff in a mixture — once it's separated out, you can often identify the components. **Paper chromatography** and **thin-layer chromatography** are two types of chromatography.

How does chromatography work?

All types of chromatography have the same basic set up:

- A **mobile phase** — where the molecules can move. In both paper and thin-layer chromatography, the mobile phase is a liquid solvent.
- A **stationary phase** — where the molecules can't move. In paper chromatography, the stationary phase is a piece of chromatography paper. In thin-layer chromatography, the stationary phase is a thin (0.1-0.3 mm) layer of solid, e.g. silica gel, on a glass or plastic plate (called a TLC plate).

All types of chromatography work using the same basic principle:

- The mobile phase moves through or over the stationary phase.
- The components in the mixture spend different amounts of time in the mobile phase and the stationary phase.
- The components that spend longer in the mobile phase travel faster or further. The time spent in the different phases is what separates out the components of the mixture (see Figure 1).

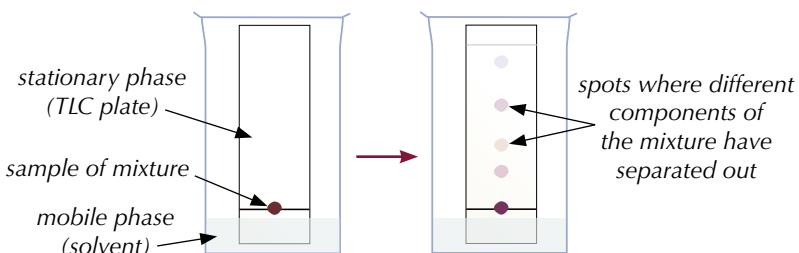


Figure 1: An example of a TLC plate before and after it has been allowed to run.

Investigating the pigments in leaves

All plants contain several different photosynthetic pigments in their leaves. Each pigment absorbs a different wavelength of light, so having more than one type of pigment increases the range of wavelengths of light that a plant can absorb. In addition to photosynthetic pigments, some plants also have other pigments in their leaves, which play other essential roles, e.g. protecting the leaves from excessive UV radiation. Different species of plants contain different proportions and mixtures of pigments.

REQUIRED PRACTICAL **7**

A sample of pigments can be extracted from the leaves of a plant and separated using paper or thin-layer chromatography. You can then identify the pigments present in the sample by calculating their **R_f values**. An R_f value is the distance a substance has moved through the stationary phase in relation to the solvent. Each pigment has a specific R_f value, under specific conditions, which can be looked up in a database.

Example

This example shows you how to use TLC to compare the pigments present in shade-tolerant plants (e.g. hostas) and shade-intolerant plants (e.g. chrysanthemums). Make sure you're wearing a lab coat, eye protection and gloves before you start.

1. Grind up several leaves from the shade-tolerant plant you're investigating with some anhydrous sodium sulfate, then add a few drops of propanone.
2. Transfer the liquid to a test tube, add some petroleum ether and gently shake the tube. Two distinct layers will form in the liquid — the top layer is the pigments mixed in with the petroleum ether.
3. Transfer some of the liquid from the top layer into a second test tube with some anhydrous sodium sulfate.
4. Draw a horizontal pencil line near the bottom of a TLC plate. Build up a concentrated spot of the liquid from step 3 on the line by applying several drops, ensuring each one is dry before the next is added. This is the point of origin.
5. Once the plate is completely dry, put the plate into a small glass container with some prepared solvent (e.g. a mixture of propanone, cyclohexane and petroleum ether) — just enough so that the point of origin is a little bit above the solvent. Put a lid on the container and leave the plate to develop. As the solvent spreads up the plate, the different pigments move with it, but at different rates — so they separate.
6. When the solvent has nearly reached the top, take the plate out and mark the solvent front (the furthest point the solvent has reached) with a pencil and leave the plate to dry in a well-ventilated place.
7. There should be several new coloured spots on the chromatography plate between the point of origin and the solvent front. These are the separated pigments. You can calculate their R_f values and look them up in a database to identify what the pigments are. You can calculate the R_f value using this formula:

$$R_f \text{ value} = \frac{B}{A} = \frac{\text{distance travelled by spot}}{\text{distance travelled by solvent}}$$

8. Repeat the process for the shade-intolerant plant you're investigating and compare the pigments present in their leaves.

You may find that the mixture of pigments in the leaves of the shade-tolerant plant is quite different compared to the shade-intolerant plant. One way that shade-tolerant plants can adapt to the light conditions in their environment is by possessing a different proportion of photosynthetic pigments, which allows the plant to make the best use of the light available to it. The mixture of non-photosynthetic pigments is also likely to be different. For example, the chloroplasts of shade-tolerant plants are adapted for photosynthesis in low light conditions, but really sensitive to higher levels of light. These plants sometimes produce dark red and purple pigments called anthocyanins, which are thought to protect their chloroplasts from brief exposure to higher light levels.

Tip: Make sure you carry out a risk assessment before you do this experiment. Be especially aware of the hazards involved with using propanone, petroleum ether and the chromatography solvent, which are toxic and highly flammable.

Tip: It's best to do steps 2 and 5 in a fume cupboard as the chemicals used are volatile (evaporate easily) and the vapours are hazardous.

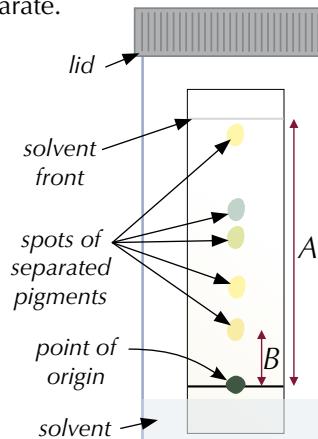


Figure 3: Diagram showing plant pigments separated by thin layer chromatography.

Tip: Some plants (including chrysanthemums) can cause allergies in some people. Handle them carefully.

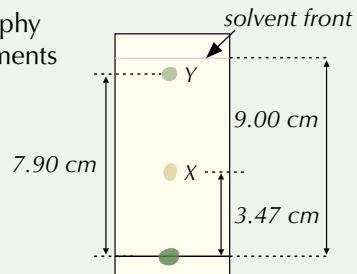
Tip: You could use the same technique to investigate the pigments in different coloured leaves. Follow the protocol described here, but change the leaves you use.

Tip: R_f values are always between 0 and 1.

Tip: The stationary phase and solvent that you use will affect the R_f value. If you're looking up R_f values, you need to check that they were recorded under the same conditions as your experiment.

Practice Question — Application

- Q1 A scientist uses thin-layer chromatography to separate out the photosynthetic pigments from a mixture obtained from plant leaves. The chromatogram that he produces is shown on the right.
- Explain why the different pigments separate as they travel up the plate.
 - Calculate the R_f value of spot X.



Investigating the activity of dehydrogenase in chloroplasts

REQUIRED PRACTICAL 8

In photosystem I, during the light-dependent stage of photosynthesis, NADP acts as an electron acceptor and is reduced (see page 267). The reaction is catalysed by a dehydrogenase enzyme. The activity of this enzyme can be investigated by adding a **redox indicator dye** to extracts of chloroplasts. Like NADP, the dye acts as an electron acceptor and gets reduced by the dehydrogenase in the chloroplasts. As the dye gets reduced, you'll see a colour change. For example, the dye DCPIP changes from blue to colourless when it gets reduced.

You can measure the rate of the dehydrogenase activity by measuring the rate at which DCPIP loses its blue colour. To do this, you need a **colorimeter**. A colorimeter measures how much light a solution absorbs when a light source is shone directly through it. A coloured solution absorbs more light than a colourless solution.

Tip: There's more on using a colorimeter on pages 100 and 365.

Tip: Make sure you're aware of all the hazards involved in this experiment, and any safety precautions you need to take to minimise them, before you start.

Tip: A centrifuge is a machine that spins samples really quickly. The resulting force separates out the components of your sample. Always make sure your centrifuge is balanced by placing tubes of equal weight opposite each other in the rotor.

Example

This example shows you how to investigate the effect of light intensity on dehydrogenase activity in extracts of chloroplasts. It uses a bench lamp as a light source and involves placing tubes of chloroplast extract mixed with DCPIP at a range of different distances from the light source. Light intensity should decrease with increasing distance from the lamp. You'll need to choose the distances you're going to investigate (e.g. 15 cm, 30 cm and 45 cm) before you start.

- Cut a few leaves (spinach works well) into pieces. Remove any tough stalks.
- Using a pestle and mortar, grind up the leaf pieces with some chilled isolation solution (a solution of sucrose, potassium chloride and phosphate buffer at pH 7). Filter the liquid you make into a beaker through a funnel lined with muslin cloth.
- Transfer the liquid to centrifuge tubes and centrifuge them at high speed for 10 minutes. This will make the chloroplasts gather at the bottom of each tube in a 'pellet' (see Figure 5).
- Get rid of the liquid from the top of the tubes, leaving the pellets in the bottom.

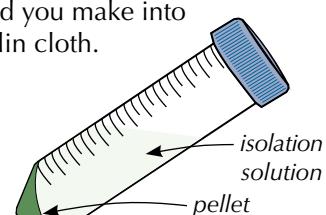


Figure 5: Diagram showing a pellet of chloroplasts after centrifugation of a leaf extract.

5. Re-suspend the pellets in fresh, chilled isolation solution. This is your chloroplast extract. Store it on ice for the rest of the experiment.
6. Set up a colorimeter with a red filter and zero it using a cuvette (a cuboid-shaped vessel used in colorimeters) containing the chloroplast extract and distilled water.
7. Set up a test tube rack at a set distance from a bench lamp. Switch the lamp on.
8. Put a test tube in the rack, add a set volume of chloroplast extract to the tube and a set volume of DCPIP. Mix the contents of the tube together.
9. Immediately take a sample of the mixture from the tube and add it to a clean cuvette. Then place the cuvette in your colorimeter and record the absorbance. Do this every 2 minutes for the next ten minutes.
10. Repeat steps 7 to 9 for each distance under investigation.
11. You should also check whether the absorbance changes at each distance in two negative control tubes. The first should contain only DCPIP and chilled isolation solution (no chloroplast extract). The second should contain both DCPIP and chloroplast extract, but it should be wrapped in tin foil (so no light reaches the contents of the tube). No change in absorbance should be seen for these two controls.

If dehydrogenase activity is taking place, the absorbance will decrease as the DCPIP gets reduced and loses its blue colour. The faster the absorbance decreases, the faster the rate of dehydrogenase activity.

You can plot a graph of absorbance against time for each distance from the light source. Then compare your results to determine how light intensity affects the rate of the dehydrogenase enzyme.

Tip: You can use a similar method to investigate the effects of other factors on dehydrogenase activity in chloroplasts, e.g. temperature and photosynthetic inhibitors.

Tip: The first negative control tube should show that the chloroplast extract is needed to make DCPIP change colour. The second negative control tube should show that light is needed to make DCPIP change colour.

Practice Questions — Fact Recall

- Q1 Explain how chromatography can be used to separate the components in a mixture.
- Q2 Explain how DCPIP acts as a redox indicator dye.
- Q3 Describe how you could prepare an extract of chloroplasts from spinach leaves in order to investigate dehydrogenase activity.

Tip: Ideally, you'd repeat the experiment at each distance at least three times and plot the mean absorbance at each 2 minute interval. Alternatively, if your classmates are doing exactly the same experiment as you, you might be able to pool your results to obtain repeat readings.

Learning Objectives:

- Know that respiration produces ATP.
- Know that respiratory substrates other than glucose include the breakdown products of lipids and amino acids, which enter the Krebs cycle.
- Know that glycolysis is the first stage of anaerobic and aerobic respiration. It occurs in the cytoplasm and is an anaerobic process. It involves the following stages:
 - phosphorylation of glucose to glucose phosphate, using ATP,
 - production of triose phosphate,
 - oxidation of triose phosphate to pyruvate with a net gain of ATP and reduced NAD.
- Know that if respiration is aerobic, pyruvate from glycolysis enters the mitochondrial matrix by active transport.
- Know that if respiration is only anaerobic, pyruvate can be converted to ethanol or lactate using reduced NAD. The oxidised NAD produced in this way can be used in further glycolysis.

Specification Reference 3.5.2

6. Aerobic and Anaerobic Respiration

Respiration is the process that allows cells to produce ATP from glucose.

Aerobic vs anaerobic respiration

Respiration can be done aerobically (with oxygen) or anaerobically (without oxygen). Both types of respiration produce ATP, but anaerobic respiration produces less. Both also start with the process of **glycolysis**. The stages after glycolysis differ.

Mitochondria

The reactions in aerobic respiration take place in the mitochondria. You covered mitochondrial structure in Topic 2, but you might want to refresh your memory of it before you start this section — see Figure 1. The folds (cristae) in the inner membrane of the mitochondrion provide a large surface area to maximise respiration.

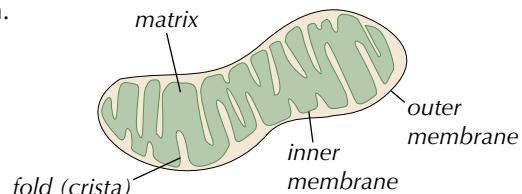


Figure 1: A mitochondrion in a nerve cell (left) and mitochondrial structure (right).

Coenzymes

As you saw in photosynthesis, a coenzyme is a molecule that aids the function of an enzyme by transferring a chemical group from one molecule to another. Coenzymes used in respiration include **NAD**, **coenzyme A** and **FAD**. NAD and FAD transfer hydrogen from one molecule to another. This means they can reduce (give hydrogen to) or oxidise (take hydrogen from) a molecule. Coenzyme A transfers acetate between molecules (see page 283).

Aerobic respiration

There are four stages in aerobic respiration:

1. Glycolysis.
2. The link reaction.
3. The Krebs cycle.
4. Oxidative phosphorylation.

The first three stages are a series of reactions. The products from these reactions are used in the final stage to produce loads of ATP. The first stage happens in the cytoplasm of cells and the other three stages take place in the mitochondria. There's more about the final three stages of aerobic respiration on pages 283-285.

Glucose can be used as a respiratory substrate in both aerobic and anaerobic respiration. However, glucose isn't the only respiratory substrate that can be used in aerobic respiration. Some products resulting from the breakdown of other molecules, such as fatty acids from lipids and amino acids from proteins, can be converted into molecules that are able to enter the Krebs cycle (usually acetyl CoA — see page 283).

Anaerobic respiration doesn't involve the link reaction, the Krebs cycle or oxidative phosphorylation. The products of glycolysis are converted to ethanol or lactate instead (see next page).

Glycolysis

Glycolysis makes **pyruvate** from glucose. Glycolysis involves splitting one molecule of glucose (with 6 carbons — 6C) into two smaller molecules of pyruvate (3C). The process happens in the cytoplasm of cells. Glycolysis is the first stage of both aerobic and anaerobic respiration and doesn't need oxygen to take place — so it's an anaerobic process.

Stages in glycolysis

There are two stages in glycolysis — phosphorylation and oxidation.

First, ATP is used to phosphorylate glucose to triose phosphate.

Phosphorylation is the process of adding phosphate to a molecule.

Then triose phosphate is oxidised, releasing ATP. Overall there's a net gain of 2 ATP and 2 reduced NAD.

1. Phosphorylation

Glucose is phosphorylated using a phosphate from a molecule of ATP. This creates 1 molecule of **glucose phosphate** and 1 molecule of ADP.

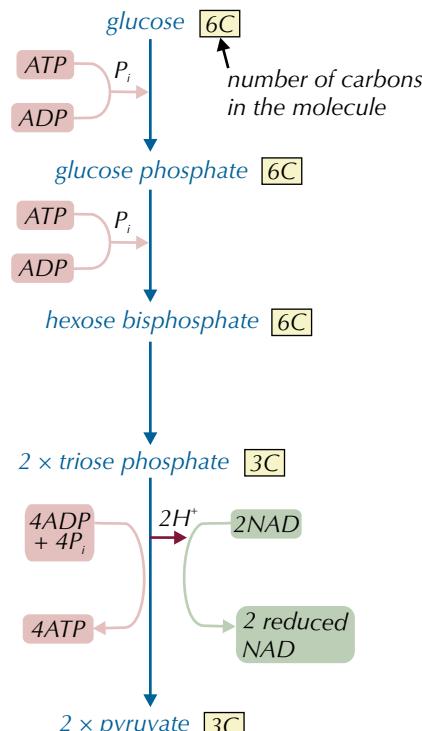
ATP is then used to add another phosphate, forming hexose bisphosphate.

Hexose bisphosphate is then split into 2 molecules of **triose phosphate**.

2. Oxidation

Triose phosphate is oxidised (loses hydrogen), forming 2 molecules of **pyruvate**. NAD collects the hydrogen ions, forming 2 **reduced NAD**.

4 ATP are produced, but 2 were used up in stage one, so there's a net gain of 2 ATP.



Exam Tip

The important thing to remember here for your exams is that 1 molecule of glucose gets phosphorylated using 1 molecule of ATP to produce 1 molecule of glucose phosphate.

Tip: Remember the first part of OILRIG, (page 264) — oxidation is loss, so when triose phosphate is oxidised it loses hydrogen.

The products of glycolysis — aerobic respiration

Here's what happens to all the products of glycolysis in aerobic respiration.

| Products from glycolysis | Where it goes |
|--------------------------|---|
| 2 reduced NAD | To oxidative phosphorylation |
| 2 pyruvate | Actively transported into the mitochondrial matrix for use in the link reaction |
| 2 ATP (net gain) | Used for energy |

Tip: Glycolysis takes place in the cytoplasm of cells because glucose can't cross the outer mitochondrial membrane. Pyruvate can cross this membrane, so the rest of the reactions in aerobic respiration occur within the mitochondria.

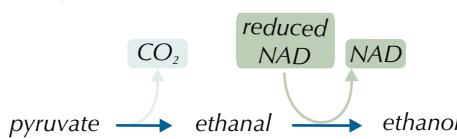
The products of glycolysis — anaerobic respiration

In anaerobic respiration, the pyruvate produced in glycolysis is converted into ethanol (alcoholic fermentation) or lactate (lactate fermentation) using reduced NAD.

Alcoholic fermentation

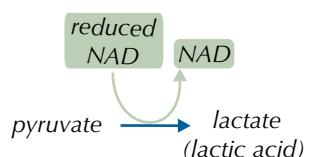
This occurs in plants and yeast.

Tip: Glycolysis only produces 2 ATP, so anaerobic respiration only produces 2 ATP. Aerobic respiration has further stages (see pages 283-285), so produces more ATP.



Lactate fermentation

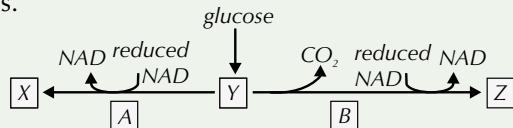
This occurs in animal cells and some bacteria.



The production of lactate or ethanol regenerates oxidised NAD. This means glycolysis can continue even when there isn't much oxygen around, so a small amount of ATP can still be produced to keep some biological process going... clever.

Practice Questions — Application

- Q1 Hexokinase is an enzyme that catalyses the production of glucose phosphate.
- Suggest and explain how hexokinase is involved in glycolysis.
 - Suggest a benefit of hexokinase being inhibited by the product of this reaction.
- Q2 The diagram below shows two possible fates of glucose in anaerobic conditions.



- What is the name of substance:
 - X?
 - Y?
 - Z?
- Which process, A or B:
 - is lactate fermentation?
 - happens in plant cells?
 - can happen in bacterial cells?

Practice Questions — Fact Recall

- Q1 What is ATP used for in glycolysis?
- Q2 What role does the coenzyme NAD play in glycolysis?
- Q3 If five molecules of glucose enter the process of glycolysis, how many molecules of pyruvate will be produced?
- Q4 During fermentation, reduced NAD is oxidised to NAD. What happens to this oxidised NAD?
- Q5 What is the final product of anaerobic respiration by animal cells?

7. Aerobic Respiration — The Mitochondrial Reactions

Aerobic respiration starts in the cytoplasm with glycolysis. The rest of aerobic respiration, starting with the link reaction, takes place in the mitochondria.

The link reaction

The link reaction converts the pyruvate produced in glycolysis (see page 281) to acetyl coenzyme A. Pyruvate is **decarboxylated**, so one carbon atom is removed from pyruvate in the form of carbon dioxide. At the same time, pyruvate is **oxidised** to form acetate and NAD is reduced to form reduced NAD. Acetate is then combined with coenzyme A (CoA) to form acetyl coenzyme A (acetyl CoA). No ATP is produced in this reaction.

How many times does the link reaction occur per glucose molecule?

Two pyruvate molecules are made for every glucose molecule that enters glycolysis. This means the link reaction and the third stage (the Krebs cycle) happen twice for every glucose molecule.

The products of the link reaction

Here's what happens to the products of two link reactions (i.e. for one glucose molecule):

| Products from two link reactions | Where it goes |
|----------------------------------|------------------------------|
| 2 acetyl coenzyme A | To the Krebs cycle |
| 2 carbon dioxide | Released as a waste product |
| 2 reduced NAD | To oxidative phosphorylation |

The Krebs cycle

The Krebs cycle produces reduced coenzymes and ATP. It involves a series of oxidation-reduction reactions, which take place in the matrix of the mitochondria. The cycle happens once for every pyruvate molecule.

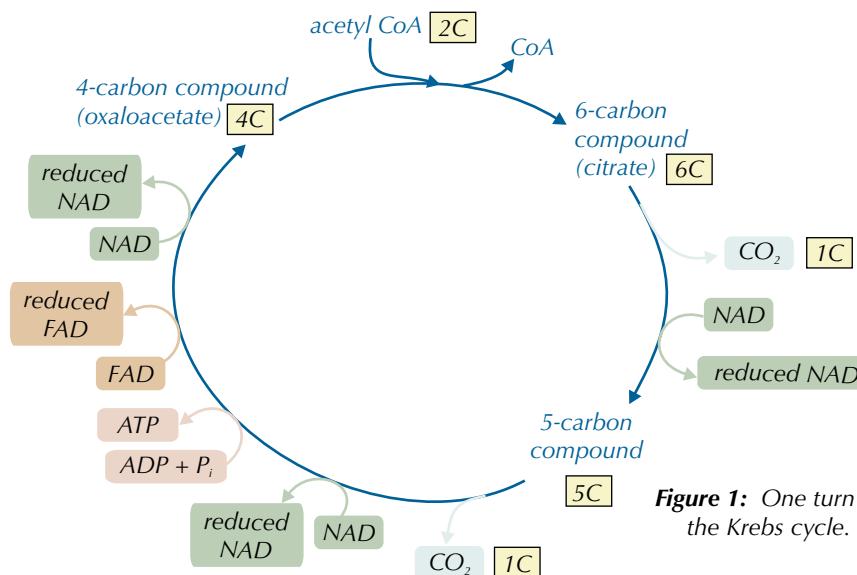


Figure 1: One turn of the Krebs cycle.

Learning Objectives:

- Understand aerobic respiration in such detail as to show that:
 - pyruvate is oxidised to acetate, producing reduced NAD in the process,
 - acetate combines with coenzyme A in the link reaction to produce acetylcoenzyme A,
 - acetylcoenzyme A reacts with a four-carbon molecule, releasing coenzyme A and producing a six-carbon molecule that enters the Krebs cycle,
- in a series of oxidation-reduction reactions, the Krebs cycle generates reduced coenzymes and ATP by substrate-level phosphorylation, and carbon dioxide is lost,
- synthesis of ATP by oxidative phosphorylation is associated with the transfer of electrons down the electron transfer chain and passage of protons across inner mitochondrial membranes and is catalysed by ATP synthase embedded in these membranes (chemiosmotic theory).

Specification Reference 3.5.2

Tip: In respiration carbon dioxide is produced in the link reaction and the Krebs cycle.

Tip: Coenzyme A transfers acetate between molecules (see page 280 for a reminder on coenzymes in respiration).

Tip: Dehydrogenation is the removal of hydrogen from a molecule.

Tip: Reduced NAD and reduced FAD may also be written as NADH and FADH_2 . Don't worry, they still mean the same thing.

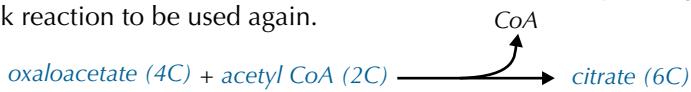
Tip: The table only shows the products of one turn of the Krebs cycle. The cycle turns twice for one glucose molecule, so one glucose molecule produces twice as much as what's shown in the table.

Tip: Remember that the Krebs cycle is just that... a cycle — some of its products need to be recycled for the process to continue.

Here's what happens at each stage in the Krebs cycle:

1. Formation of a 6-carbon compound

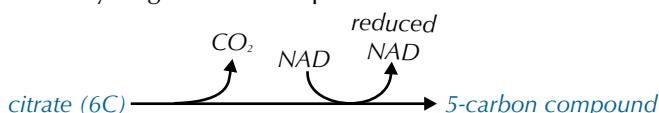
Acetyl CoA from the link reaction combines with a four-carbon molecule (oxaloacetate) to form a six-carbon molecule (citrate). Coenzyme A goes back to the link reaction to be used again.



2. Formation of a 5-carbon compound

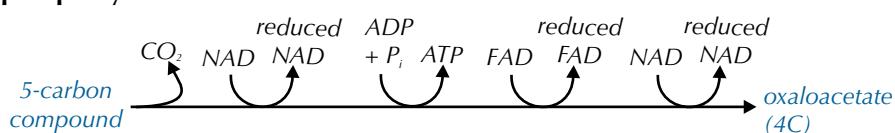
The six-carbon citrate molecule is converted to a five-carbon molecule.

Decarboxylation occurs, where carbon dioxide is removed. **Dehydrogenation** also occurs. The hydrogen is used to produce reduced NAD from NAD.



3. Regeneration of oxaloacetate

The five-carbon molecule is then converted to a four-carbon molecule. (There are some intermediate compounds formed during this conversion, but you don't need to know about them.) Decarboxylation and dehydrogenation occur, producing one molecule of reduced FAD and two of reduced NAD. ATP is produced by the direct transfer of a phosphate group from an intermediate compound to ADP. When a phosphate group is directly transferred from one molecule to another it's called **substrate-level phosphorylation**. Citrate has now been converted into oxaloacetate.



The products of the Krebs cycle

Some products of the Krebs cycle are reused, some are released and others are used for the next stage of respiration — oxidative phosphorylation.

| Product from one Krebs cycle | Where it goes |
|------------------------------|---|
| 1 coenzyme A | Reused in the next link reaction |
| Oxaloacetate | Regenerated for use in the next Krebs cycle |
| 2 carbon dioxide | Released as a waste product |
| 1 ATP | Used for energy |
| 3 reduced NAD | To oxidative phosphorylation |
| 1 reduced FAD | To oxidative phosphorylation |

Practice Questions — Application

Q1 The diagram below shows part of the Krebs cycle:



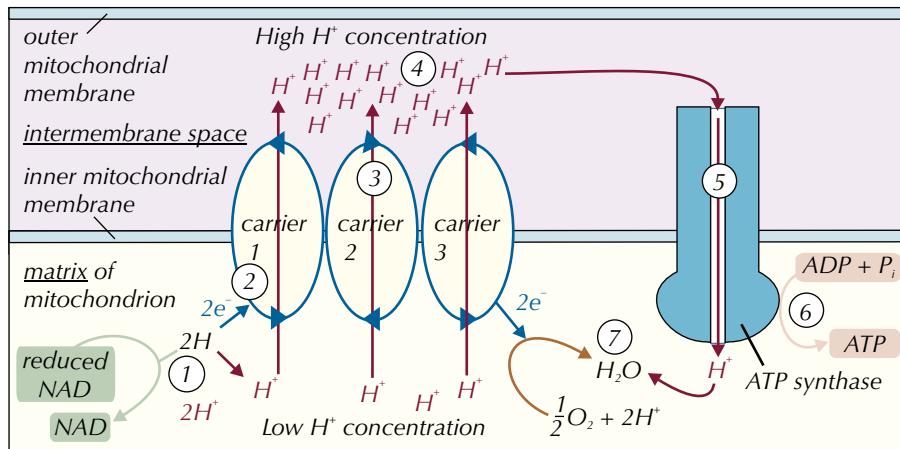
- How many carbon atoms do oxaloacetate and citrate each have?
- What happens to turn the 5C-intermediate back into oxaloacetate?

Q2 If six molecules of glucose were respired, how many molecules of CO_2 would be produced from the Krebs cycle?

Q3 Fats can be broken down and converted into acetyl coenzyme A. Explain how this allows fats to be respired.

Oxidative phosphorylation

Oxidative phosphorylation is the process where the energy carried by electrons, from reduced coenzymes (reduced NAD and reduced FAD), is used to make ATP. (The whole point of the previous stages is to make reduced NAD and reduced FAD for the final stage.) Oxidative phosphorylation involves the **electron transport chain** and **chemiosmosis** (see below).



The numbers of the steps below correspond to the circled numbers in the diagram above.

- Hydrogen atoms are released from reduced NAD and reduced FAD as they're oxidised to NAD and FAD. The hydrogen atoms split into protons (H⁺) and electrons (e⁻).
- The electrons move down the electron transport chain (made up of electron carriers), losing energy at each carrier (see Figure 2).
- This energy is used by the electron carriers to pump protons from the mitochondrial matrix into the intermembrane space (the space between the inner and outer mitochondrial membranes).
- The concentration of protons is now higher in the intermembrane space than in the mitochondrial matrix — this forms an electrochemical gradient (a concentration gradient of ions).
- Protons then move down the electrochemical gradient, back across the inner mitochondrial membrane and into the mitochondrial matrix, via ATP synthase (which is embedded in the inner mitochondrial membrane). This movement drives the synthesis of ATP from ADP and inorganic phosphate (P_i).
- This process of ATP production driven by the movement of H⁺ ions across a membrane (due to electrons moving down an electron transport chain) is called chemiosmosis (which is described by the chemiosmotic theory).
- In the mitochondrial matrix, at the end of the transport chain, the protons, electrons and oxygen (from the blood) combine to form water. Oxygen is said to be the **final electron acceptor**.

Tip: The regenerated coenzymes from the electron transport chain are reused in the Krebs cycle.

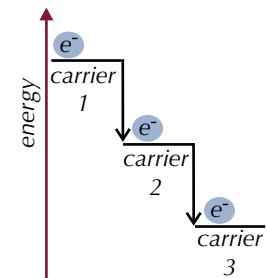


Figure 2: As electrons move down the electron transport chain, they lose energy.

Tip: The job of a carrier is to transfer electrons. When a carrier receives electrons it's reduced and when it passes on electrons it becomes oxidised again.

Exam Tip

Don't write that protons move into or out of the inner mitochondrial membrane — they move across it.

Aerobic respiration and ATP

As you know, oxidative phosphorylation makes ATP using energy from the reduced coenzymes — 2.5 ATP are made from each reduced NAD and 1.5 ATP are made from each reduced FAD.

Tip: The number of ATP produced per reduced NAD or reduced FAD was thought to be 3 and 2, but newer research has shown that the figures are nearer 2.5 and 1.5.

Tip: For each molecule of glucose, 28 molecules of ATP are produced by oxidative phosphorylation (i.e. that's the ATP made from reduced NAD and reduced FAD).

Tip: Don't forget oxygen's role in respiration. It's the final electron acceptor in the electron transport chain in oxidative phosphorylation (see previous page).

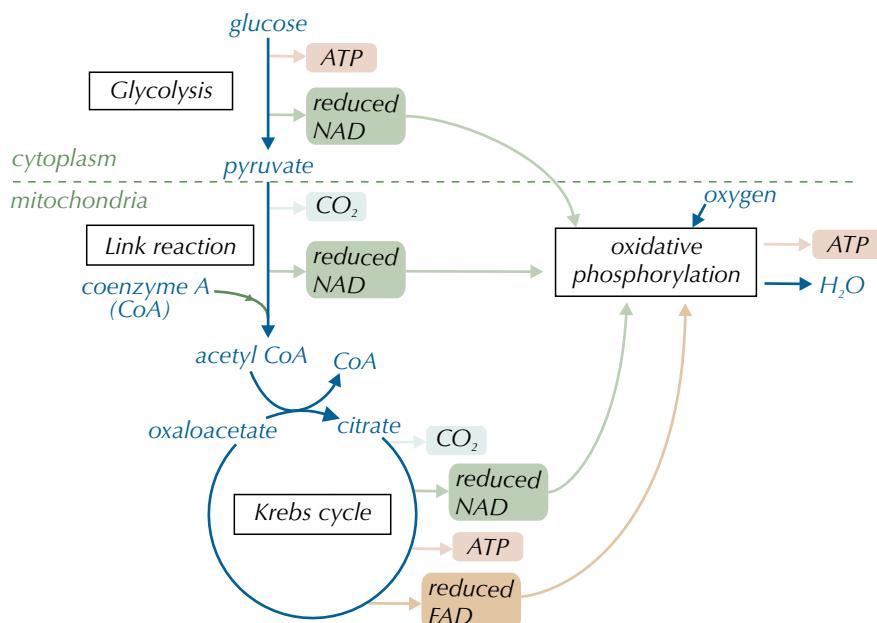
Tip: Remember that the whole purpose of respiration is to produce ATP to fuel biological processes. That's why it's happening continuously in plant and animal cells.

The table below shows that a cell can make 32 ATP from 1 molecule of glucose in aerobic respiration. (Remember, 1 molecule of glucose produces 2 pyruvate, so the link reaction and Krebs cycle happen twice.)

| Stage of respiration | Molecules produced | Number of ATP molecules |
|------------------------------|--------------------|-------------------------|
| Glycolysis | 2 ATP | 2 |
| Glycolysis | 2 reduced NAD | $2 \times 2.5 = 5$ |
| Link Reaction ($\times 2$) | 2 reduced NAD | $2 \times 2.5 = 5$ |
| Krebs cycle ($\times 2$) | 2 ATP | 2 |
| Krebs cycle ($\times 2$) | 6 reduced NAD | $6 \times 2.5 = 15$ |
| Krebs cycle ($\times 2$) | 2 reduced FAD | $2 \times 1.5 = 3$ |
| | | Total ATP = 32 |

Aerobic respiration summary

Glycolysis, the link reaction and the Krebs cycle are basically a series of reactions which produce ATP, reduced NAD, reduced FAD and CO_2 . The reduced coenzymes (NAD and FAD) are then used in oxidative phosphorylation, to produce loads more ATP. The overall process is shown below:



Mitochondrial diseases

ATP production can be affected by **mitochondrial diseases**. Mitochondrial diseases affect the functioning of mitochondria. They can affect how proteins involved in oxidative phosphorylation or the Krebs cycle function, reducing ATP production. This may cause anaerobic respiration to increase, to try and make up some of the ATP shortage. This results in lots of lactate being produced, which can cause muscle fatigue and weakness. Some lactate will also diffuse into the bloodstream, leading to high lactate concentrations in the blood.

Practice Questions — Application

- Q1 Antimycin A inhibits carrier 2 in the electron transport chain of oxidative phosphorylation.
- If antimycin A was added to isolated mitochondria, what state (oxidised or reduced) would carriers 1 and 3 be in after its addition? Explain your answers.
 - Suggest why antimycin A can be used as a fish poison.
- Q2 Dicyclohexylcarbodiimide (DCC) is an inhibitor that binds to ATP synthase and prevents protons moving through it. When mitochondria are treated with DCC they stop synthesising ATP. Explain how this provides evidence for the chemiosmotic theory.

Practice Questions — Fact Recall

- Q1 a) In the link reaction, pyruvate is converted into acetate. Describe how this happens.
- b) The second stage of the link reaction relies on coenzyme A. What is the role of coenzyme A in the link reaction?
- c) State what happens to the products of the link reaction.
- Q2 In one turn of the Krebs cycle:
- how many molecules of CO_2 are released, and where are they released from?
 - how many molecules of reduced FAD are made?
- Q3 During the Krebs cycle ATP is produced by the direct transfer of a phosphate group from an intermediate compound to ADP. What name is given to this process?
- Q4 After each turn of the Krebs cycle, what happens to:
- coenzyme A?
 - oxaloacetate?
- Q5 During oxidative phosphorylation, what happens to electrons as they move down the electron transport chain?
- Q6 What is said to be the final electron acceptor in oxidative phosphorylation?
- Q7 Give one example of a decarboxylation reaction in respiration.
- Q8 Draw out the table below and fill it in with crosses to show where the following substances are made in respiration.

Exam Tip

You really need to know this stuff for your exam. If you find you're struggling to answer a question go back to the relevant page and make sure you really understand what's going on.

| Substance | Glycolysis | Link reaction | Krebs cycle | Oxidative phosphorylation |
|---------------------------------|-------------------|----------------------|--------------------|----------------------------------|
| <i>ATP</i> | | | | |
| <i>reduced NAD</i> | | | | |
| <i>reduced FAD</i> | | | | |
| <i>CO_2</i> | | | | |

Learning Objective:

- Be able to carry out an investigation into the effect of a named variable on the rate of respiration of cultures of single-celled organisms (Required Practical 9).

Specification Reference 3.5.2

Tip: Make sure you think about and address all the risks involved in the experiments on pages 288-290 before you carry them out.

Tip: A buffer solution is able to resist changes in pH when small amounts of acid or alkali are added. You can get acidic buffers (with a pH of less than 7) and alkaline buffers (with a pH of more than 7).

Tip: The yeast will only respire aerobically until the oxygen trapped in the tube is all used up. If you wanted to run the experiment for more time or with more yeast or glucose, you could use a conical flask that can trap more oxygen.

Tip: To calculate the rate of CO_2 production, divide the total volume of CO_2 produced at a particular temperature by the number of minutes the apparatus was left for.

8. Respiration Experiments

These pages give you some experiments that you could use to carry out investigations into respiration.

Investigating factors affecting respiration in single-celled organisms

REQUIRED PRACTICAL 9

Yeast are single-celled organisms that can be grown in culture. They can respire aerobically when plenty of oxygen is available and anaerobically when oxygen isn't available. Both aerobic and anaerobic respiration in yeast produce CO_2 (see page 261). So the rate of CO_2 production gives an indication of the yeast's respiration rate. One way to measure CO_2 production is by using a gas syringe to collect the CO_2 .

The methods below and on the next page show you how to investigate the effects of temperature on both aerobic and anaerobic respiration in yeast. You'll need to decide what temperatures you're going to investigate before you start (e.g. 10 °C, 20 °C and 25 °C).

Example — Aerobic Respiration

- Put a known volume and concentration of substrate solution (e.g. glucose) in a test tube. Add a known volume of buffer solution to keep the pH constant. (Choose the optimum pH for the yeast you're testing — usually 4-6.)
- Place the test tube in a water bath set to one of the temperatures being investigated. Leave it there for 10 minutes to allow the temperature of the substrate to stabilise.
- Add a known mass of dried yeast (e.g. *Saccharomyces cerevisiae*) to the test tube and stir for two minutes.
- After the yeast has dissolved into the solution, put a bung with a tube attached to a gas syringe in the top of the test tube. The gas syringe should be set to zero.
- Start a stopwatch as soon as the bung has been put in the test tube.
- As the yeast respire, the CO_2 formed will travel up the tube and into the gas syringe, which is used to measure the volume of CO_2 released.
- At regular time intervals (e.g. every minute), record the volume of CO_2 that is present in the gas syringe. Do this for a set amount of time (e.g. 10 minutes).
- A negative control experiment should also be set up at each temperature, where no yeast is present. No CO_2 should be formed without the yeast.
- Repeat the experiment three times at each temperature you're investigating. Use your data to calculate the mean rate of CO_2 production at each temperature.

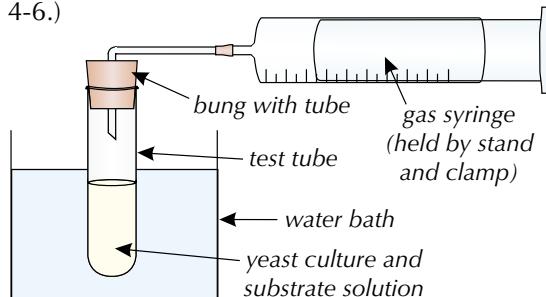


Figure 1: Diagram showing how apparatus can be set up to measure aerobic respiration in yeast.

Example — Anaerobic Respiration

- Set up the apparatus according to steps 1-3 of the experiment on the previous page.
- After the yeast has dissolved into the substrate solution, trickle some liquid paraffin down the inside of the test tube so that it settles on and completely covers the surface of the solution. This will stop oxygen getting in, which will force the yeast to respire anaerobically.
- Put a bung, with a tube attached to a gas syringe, in the top of the test tube. The gas syringe should be set to zero.
- Perform steps 5-9 from the method on the previous page.

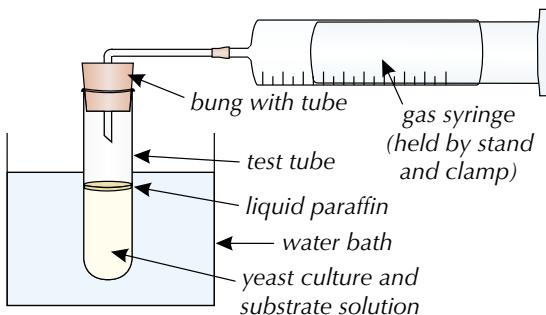


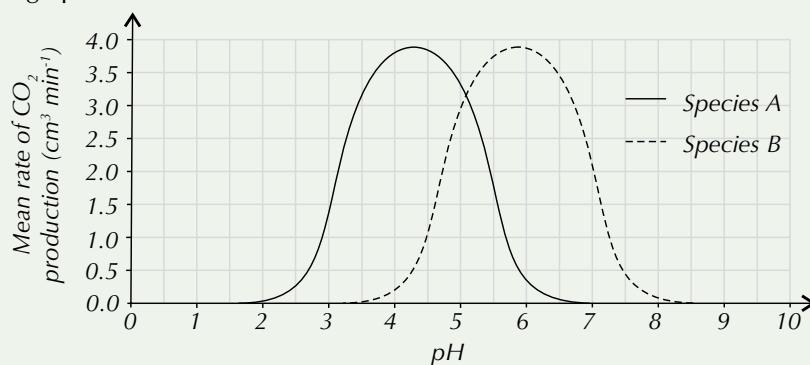
Figure 2: Diagram showing how apparatus can be set up to measure anaerobic respiration in yeast.

You can also easily adapt these methods to investigate the effects of other variables, such as substrate concentration and the use of different respiratory substrates (e.g. sucrose) on the respiration rate.

Just remember that you should only change one variable at a time (the independent variable, see page 1). All the other variables that could affect your results need to be controlled (kept the same) or your results won't be valid.

Practice Question — Application

- Q1 A scientist is investigating the effect of pH on aerobic respiration in two different species of yeast. The mean rate of CO_2 production is indicative of the respiration rate. Her results are shown in the graph below.



- Describe an experiment the scientist could have done to obtain the results shown in the graph.
- The results show that each species has a different optimum pH. Suggest an explanation for this.
- At pH 5.5, how much faster is the mean rate of CO_2 production by species B than species A? Give your answer as a percentage.
- The scientist also carried out the same experiment using boiled yeast of each species. Explain why.

Tip: To test that the gas produced is definitely CO_2 , connect the yeast and substrate solution to a test tube of limewater rather than a gas syringe. The limewater will turn cloudy in the presence of CO_2 .

Tip: There are other ways of measuring the rate of respiration in yeast. For example, you could use a redox indicator dye (e.g. methylene blue) and a colorimeter to measure the rate of aerobic respiration (the method is similar to the one used in the photosynthesis experiment on pages 278-279). The dye takes the place of electron acceptors in oxidative phosphorylation.

Tip: Before doing this experiment, you need to think about the ethical issues involved, as well as any safety issues. You must treat the woodlice with respect and ensure that they're not harmed or distressed unnecessarily.

Tip: Wear eye protection and gloves when working with potassium hydroxide, and make sure that the woodlice don't come into contact with it.



Figure 4: A respirometer set up to measure the rate of respiration by germinating peas (left). Glass beads are being used as a control (right).

Using a respirometer to measure oxygen consumption

Respirometers can be used to indicate the rate of aerobic respiration by measuring the amount of oxygen consumed by an organism over a period of time. The example below shows how a respirometer can be used to measure the respiration rate of woodlice. You could also use it to measure the respiration rate of other small organisms or of plant seeds.

Example

1. The apparatus is set up as shown in Figure 3, partially submerged in a water bath at 15 °C to provide the optimum temperature for the woodlice and therefore, the optimum temperature for the enzymes involved in their respiration.

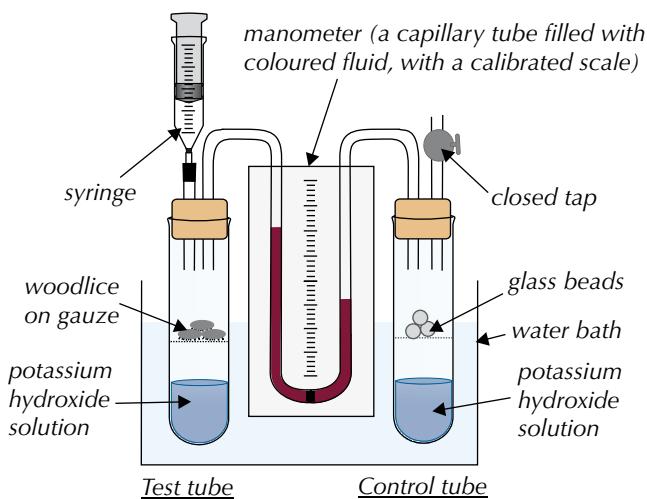


Figure 3: Diagram showing how a respirometer can be set up to measure oxygen consumption.

2. The control tube is set up in exactly the same way as the woodlouse tube, except that the woodlice are substituted with glass beads of the same mass.
3. For ten minutes, the tap is left open and the syringe is removed to allow the apparatus to equilibrate (accounting for any expansion that might cause the pressure to change inside) and the respiration rate of the woodlice to stabilise in their new environment.
4. When the ten minutes is up, the tap is closed and the syringe is attached.
5. The syringe is used to reset the manometer, so that the ends of the fluid are at the same level on either side of the 'U' and the reading from the volume scale on the syringe (usually in cm³) is recorded.
6. As respiration occurs, the volume of the air in the test tube containing woodlice will decrease, due to the oxygen consumed during respiration (all the CO₂ produced is absorbed by the potassium hydroxide).
7. The decrease in the volume of the air will reduce the pressure in the test tube, causing the coloured fluid in the capillary tube of the manometer to move towards it.

8. After leaving the apparatus to run for a set period of time (e.g. 10 minutes), the syringe is used to reset the manometer and the reading on the syringe's volume scale is recorded again. The difference between this figure and the figure taken at the start of the experiment is the oxygen consumption for this time period. You can use this to calculate a rate of respiration.
9. To check the precision of the results, the experiment is repeated and a mean volume of O_2 is calculated.

Tip: Oxygen consumption can also be calculated by recording the movement of the fluid in the manometer, read from the scale on the manometer itself.

Practice Questions — Fact Recall

- Q1 If you were measuring anaerobic respiration in yeast, why would you add a layer of liquid paraffin to the yeast solution in the test tube before sealing the tube with a rubber bung?
- Q2 Suggest a negative control experiment that could be included when measuring the rate of respiration of yeast in a test tube.
- Q3 If you were using a respirometer to measure the oxygen consumed by germinating peas with a mass of 10 g, what mass of glass beads would you have in the control tube?

Section Summary

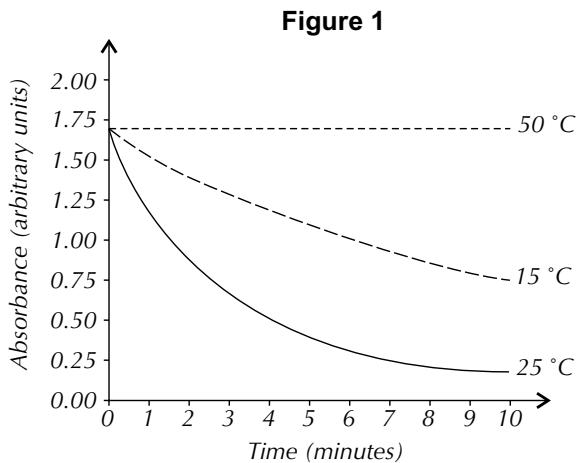
Make sure you know...

- That ATP is the immediate source of energy in a cell. It is used to carry out biological processes.
- That the compensation point in plants is the point when the rate of photosynthesis exactly matches the rate of respiration and how to identify the compensation point of a plant from a graph.
- That photosynthesis (where plants convert light energy into chemical energy in the form of glucose) has two stages — the light-dependent reaction and the light-independent reaction.
- That the light-dependent reaction includes non-cyclic photophosphorylation and cyclic photophosphorylation. In both processes, light energy is absorbed by the chlorophyll in photosystems and used to excite electrons, releasing them from the chlorophyll (photoionisation). As the electrons move down the electron transport chain they lose energy, which is used to generate a proton gradient across the thylakoid membrane. The subsequent movement of protons down their concentration gradient is used to produce ATP. In non-cyclic photophosphorylation, reduced NADP is also produced.
- That the process of electrons flowing down the electron transport chain and creating a proton gradient across the membrane to drive ATP synthesis is called chemiosmosis. It's described by the chemiosmotic theory.
- That the photolysis of water is the splitting of water using light and that it produces protons, electrons and oxygen. It happens in non-cyclic photophosphorylation.
- That cyclic photophosphorylation only produces small amounts of ATP and doesn't produce any reduced NADP or oxygen.
- That in the light-independent reaction carbon dioxide (CO_2) enters the Calvin cycle, where it is combined with ribulose bisphosphate (RuBP) to form two molecules of glycerate 3-phosphate (GP). This reaction is catalysed by the enzyme rubisco. These two molecules of GP are then reduced to two molecules of triose phosphate (TP), using ATP and reduced NADP from the light-dependent reaction. Five out of every six molecules of TP are used to regenerate RuBP (allowing the Calvin cycle to continue) while the remaining TP is used to produce organic substrates such as carbohydrates, lipids and proteins.

- That a limiting factor is a variable that can slow down the rate of a reaction. The limiting factors of photosynthesis are light intensity, temperature and carbon dioxide concentration.
- That agricultural growers create ideal conditions of light intensity, carbon dioxide concentration and temperature in glasshouses, so that these factors are less likely to limit photosynthesis and that crop yield is increased.
- How to interpret data on agricultural processes used to overcome limiting factors in photosynthesis, e.g. using heaters in glasshouses.
- How to use chromatography to separate the pigments in the leaves of plants to allow you to compare the pigments present in shade-tolerant and shade-intolerant plants or in different coloured leaves (Required Practical 7).
- How to use a redox indicator dye to investigate the effect of a named factor (e.g. light intensity) on the activity of dehydrogenase enzyme in extracts of chloroplasts (Required Practical 8).
- That both aerobic and anaerobic respiration produce ATP, and both begin with glycolysis.
- That respiratory substrates other than glucose can be used for aerobic respiration, including the breakdown products of lipids and amino acids, which enter the Krebs cycle.
- The four stages of aerobic respiration — glycolysis (which happens in the cytoplasm), the link reaction, the Krebs cycle and oxidative phosphorylation (the last three stages happen in the mitochondria).
- That in glycolysis, ATP is used to phosphorylate glucose to glucose phosphate. Glucose phosphate is then converted into hexose bisphosphate, using ATP to add another phosphate. Hexose bisphosphate is then split into two molecules of triose phosphate. Triose phosphate is then oxidised to pyruvate. There is a net gain of two ATP and two reduced NAD, per molecule of glucose.
- That in anaerobic respiration pyruvate (from glycolysis) is converted to ethanol or lactate. Only two ATP per molecule of glucose can be produced by this method and NAD is regenerated.
- That in the link reaction of aerobic respiration, pyruvate is oxidised to acetate (via decarboxylation and the reduction of NAD). Then acetate is combined with coenzyme A to form acetyl coenzyme A.
- That acetyl coenzyme A (a two-carbon molecule) combines with a four-carbon molecule (oxaloacetate) to produce a six-carbon molecule (citrate) in the first reaction of the Krebs cycle. This is followed by a series of oxidation-reduction reactions to produce reduced NAD, reduced FAD (reduced coenzymes) and ATP. CO_2 is also produced and lost in the process. The reduced coenzymes are used in oxidative phosphorylation.
- That ATP is produced in the Krebs cycle by substrate-level phosphorylation — a phosphate group is directly transferred from an intermediate molecule to ADP.
- That oxidative phosphorylation uses electrons from reduced NAD and reduced FAD to make ATP. Electrons travel down the electron transport chain, losing energy as they go. This energy is used to form a proton gradient across the inner mitochondrial membrane, which is used to make ATP by chemiosmosis (which is described by the chemiosmotic theory). Water is also produced in this process.
- How to carry out an investigation into the effect of a named variable (e.g. temperature) on the rate of respiration in cultures of single-celled organisms (Required Practical 9).

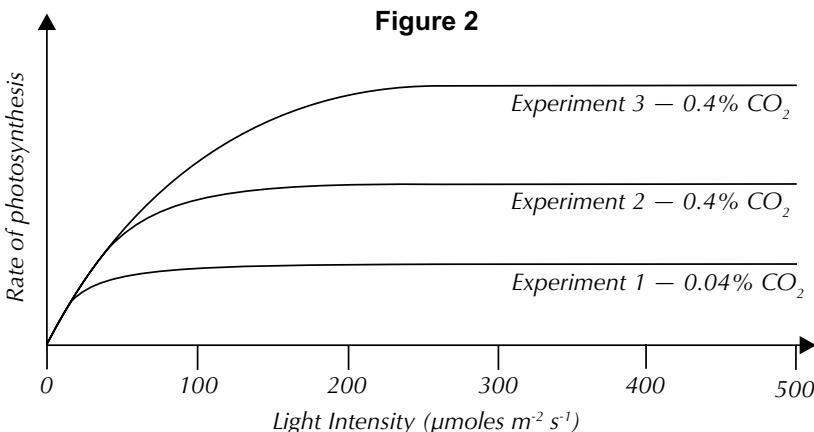
Exam-style Questions

- 1 Petite mutants are yeast cells that have mutations in genes that are important for mitochondrial function. They are called petite mutants because they grow and divide to form unusually small colonies when grown in medium with a low glucose concentration.
- 1.1 Petite mutants are unable to produce mitochondrial proteins.
Suggest how this could stop the mitochondria from producing ATP.
(1 mark)
- 1.2 Petite mutants lack functioning mitochondria but they can still produce ATP by glycolysis. Explain why.
(1 mark)
- 1.3 Triose phosphate is an intermediate compound in glycolysis.
Describe how two triose phosphate molecules are formed from a molecule of glucose.
(3 marks)
- 1.4 Describe the role of the coenzyme NAD in glycolysis.
(2 marks)
- 2 DCPIP is an artificial hydrogen acceptor that can be used to measure the rate of photosynthesis. When DCPIP is reduced it turns from blue to colourless. In the presence of NADP, DCPIP is reduced first. A scientist used DCPIP to investigate the rate of photosynthesis in plant chloroplasts at three different temperatures. DCPIP was incubated with liquid extracts of chloroplasts for 10 minutes. Every minute, the absorbance of the solution was measured. All conditions except the temperature were kept the same. The results are shown in **Figure 1**.
- 2.1 Suggest how the absorbance of the solution was measured.
(2 marks)
- 2.2 Why was measuring the absorbance of the solution over time a suitable way of indicating the rate of photosynthesis?
(3 marks)
- 2.3 Suggest why the absorbance doesn't change at 50 °C.
(3 marks)



- 3 In oxidative phosphorylation hydrogen atoms are released from reduced NAD and reduced FAD.
- 3.1 Describe the reactions in respiration in which these reduced coenzymes are produced. (5 marks)
- 3.2 The hydrogen atoms split up into hydrogen ions and electrons. Describe the movement of electrons in oxidative phosphorylation. (2 marks)
- 3.3 DNP is an uncoupler. This means it carries H⁺ ions from the intermembrane space back into the matrix of mitochondria during oxidative phosphorylation. Describe and explain the effect that DNP would have on the production of ATP in animal cells. (4 marks)
- 4 A student carried out a study into the effect of different factors on the rate of photosynthesis in a certain species of plant.
- 4.1 The student calculated the rate of photosynthesis by measuring how much oxygen was released by the plants over a period of time. Explain why this is not an accurate way of calculating the rate of photosynthesis. (2 marks)

The student carried out three experiments in his study — the results of which are shown in **Figure 2**. In each experiment the plants had an adequate supply of water.



- 4.2 What is the limiting factor of photosynthesis in experiment 2? Explain your answer. (2 marks)
- 4.3 The student extended experiment 2 by measuring the amount of RuBP and TP produced by the plant over time. After 5 minutes, the student lowered the CO_2 concentration of the plants to 0.04%. Describe and explain what effect the lowering of CO_2 concentration had on the levels of RuBP and TP in the plants. (3 marks)

1. Energy Transfer in Ecosystems

Plants get their energy from the Sun, and animals get their energy by eating plants or other animals. Some energy gets lost along the way, but you can calculate the energy that gets transferred using some nifty equations.

Ecosystem basics

An ecosystem includes all the organisms living in a particular area and all the non-living (abiotic) conditions (see page 415). In all ecosystems, there are producers — organisms that make their own food.

Examples

- In land-based ecosystems, plants (such as trees, shrubs and grasses) produce their own food through photosynthesis.
- In aquatic ecosystems, plants (such as water lilies and watercress) and algae (such as seaweeds) also produce their own food through photosynthesis.

During photosynthesis plants use energy (from sunlight) and carbon dioxide (from the atmosphere in land-based ecosystems, or dissolved in water in aquatic ecosystems) to make glucose and other sugars.

Some of the sugars produced during photosynthesis are used in respiration, to release energy for growth. The rest of the glucose is used to make other biological molecules, such as cellulose (a component of plant cell walls). These biological molecules make up the plant's **biomass** — the mass of living material. Biomass can also be thought of as the chemical energy stored in the plant.

Energy is transferred through the living organisms of an ecosystem when organisms eat other organisms, e.g. producers are eaten by organisms called primary consumers. Primary consumers are then eaten by secondary consumers and secondary consumers are eaten by tertiary consumers. This is a food chain (see page 299).

Measuring biomass

Biomass can be measured in terms of the mass of carbon that an organism contains or the dry mass of its tissue per unit area. Dry mass is the mass of the organism with the water removed. The water content of living tissue varies, so dry mass is used as a measure of biomass rather than wet mass.

To measure the dry mass, a sample of the organism is dried, often in an oven set to a low temperature. The sample is then weighed at regular intervals (e.g. every day). Once the mass becomes constant you know that all the water has been removed. The mass of carbon present is generally taken to be 50% of the dry mass.

Once you've measured the dry mass of a sample, you can scale up the result to give the dry mass (biomass) of the total population or the area being investigated. So typical units for dry mass might be kg m^{-2} .

Learning Objectives:

- Know that in any ecosystem, plants synthesise organic compounds from atmospheric, or aquatic, carbon dioxide.
- Know that most of the sugars synthesised by plants are used by the plant as respiratory substrates, and that the rest are used to make other groups of biological molecules. These biological molecules form the biomass of the plants.
- Know that biomass can be measured in terms of mass of carbon or dry mass of tissue per given area.
- Know that the chemical energy store in dry biomass can be estimated using calorimetry.
- Know that gross primary production (*GPP*) is the chemical energy store in plant biomass, in a given area or volume.
- Know that net primary production (*NPP*) is the chemical energy store in plant biomass after respiratory losses to the environment (*R*) have been taken into account, i.e.
$$NPP = GPP - R.$$
- Know that primary productivity is the rate of primary production and is measured as biomass, in a given area, in a given time.

- Know that net primary production is available for plant growth and reproduction.
- Know that net primary production is also available to other trophic levels in the ecosystem such as herbivores and decomposers.
- Know that the net production of consumers (N), such as animals, can be calculated as $N = I - (F + R)$, where I represents the chemical energy stored in ingested food, F represents the chemical energy lost to the environment in faeces and urine and R represents the respiratory losses to the environment.
- Know that the net production of consumers is also known as secondary production and that this is called secondary productivity when expressed as a rate.

Specification Reference 3.5.3

Tip: Remember, plants convert light energy to chemical energy during photosynthesis.

Tip: Photosynthesis isn't 100% efficient. Not all of the light energy absorbed by a plant will be converted to chemical energy.

Calorimetry

You can estimate the amount of chemical energy stored in biomass by burning the biomass in a **calorimeter** (see Figure 1). The amount of heat given off tells you how much energy is in it. Energy is measured in joules (J) or kilojoules (kJ).

A sample of dry biomass is burnt and the energy released is used to heat a known volume of water. The change in temperature of the water is used to calculate the chemical energy of the dry biomass.

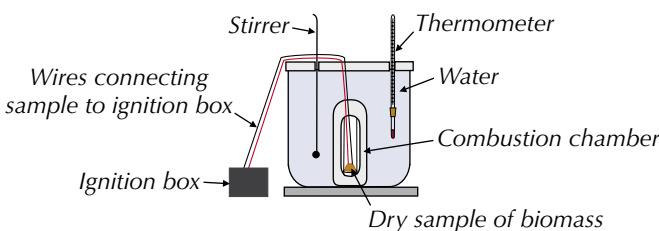


Figure 1: An example of a calorimeter being used to measure the chemical energy in biomass.

Primary production

Gross primary production (GPP) is the total amount of chemical energy converted from light energy by plants, in a given area. Approximately 50% of the gross primary production is lost to the environment as heat when the plants respire. This is called **respiratory loss (R)**. The remaining chemical energy is called the **net primary production (NPP)**. This relationship is shown by the following formula:

$$\boxed{NPP = GPP - R}$$

Often, primary production is expressed as a rate — i.e. the total amount of chemical energy (or biomass) in a given area, in a given time. Typical units might be $\text{kJ ha}^{-1} \text{yr}^{-1}$ (kilojoules per hectare per year) or $\text{kJ m}^{-2} \text{ yr}^{-1}$ (kilojoules per square metre per year). When primary production is expressed as a rate it is called **primary productivity**.

Examples — Maths Skills

- The grass in an ecosystem has a gross primary productivity of $20\ 000 \text{ kJ m}^{-2} \text{ yr}^{-1}$. It loses $8000 \text{ kJ m}^{-2} \text{ yr}^{-1}$ as heat from respiration.
Calculate the net primary productivity of the grass in this ecosystem.
net primary productivity = $20\ 000 - 8000$
 $= 12\ 000 \text{ kJ m}^{-2} \text{ yr}^{-1}$

- The net primary productivity in an area of tundra is $2800 \text{ kJ m}^{-2} \text{ yr}^{-1}$. It loses $1250 \text{ kJ m}^{-2} \text{ yr}^{-1}$ through respiration.

Calculate the gross primary productivity of the area of tundra.

- First you need to rearrange the formula:
 $NPP = GPP - R$, so $GPP = NPP + R$
- Then you can use it to calculate GPP :
gross primary productivity = $2800 + 1250$
 $= 4050 \text{ kJ m}^{-2} \text{ yr}^{-1}$

The NPP is the energy available to the plant for growth and reproduction — the energy is stored in the plant's biomass. It is also the energy available to organisms at the next stage in the food chain (the next trophic level, see page 299). These include herbivores (animals that eat the plants) and decomposers.

Net production in consumers

Consumers also store chemical energy in their biomass. Consumers get energy by ingesting plant material, or animals that have eaten plant material. However, not all the chemical energy stored in the consumers' food is transferred to the next trophic level — around 90% of the total available energy is lost in various ways. Firstly, not all of the food is eaten (e.g. plant roots, bones) so the energy it contains is not taken in. Then, of the parts that are ingested:

- Some are indigestible, so are egested as faeces. The chemical energy stored in these parts is therefore lost to the environment.
- Some energy is also lost to the environment through respiration or excretion of urine.

The energy that's left after all this is stored in the consumers' biomass and is available to the next trophic level. This energy is the consumers' **net production**. The net production of consumers can be calculated using the following formula:

$$N = I - (F + R)$$

Where:

N = Net production

I = Chemical energy in ingested food

F = Chemical energy lost in faeces and urine

R = Energy lost through respiration

Tip: There are lots of similar sounding words here. 'Ingest' means 'take in to the body'. 'Indigestible' means 'can't be digested (broken down)'. 'Egest' means 'get rid of from the body'.

Example — Maths Skills

The rabbits in an ecosystem ingest 20 000 $\text{kJ m}^{-2} \text{ yr}^{-1}$ of energy, but lose 12 000 $\text{kJ m}^{-2} \text{ yr}^{-1}$ of it in faeces and urine. They lose a further 6000 $\text{kJ m}^{-2} \text{ yr}^{-1}$ using energy for respiration. You can use this to calculate the net productivity of the rabbits:

$$\begin{aligned}\text{net productivity} &= 20\,000 - (12\,000 + 6000) \\ &= 20\,000 - 18\,000 = \mathbf{2000 \text{ kJ m}^{-2} \text{ yr}^{-1}}\end{aligned}$$

The net production of consumers can also be called **secondary production** (or secondary productivity when it's expressed as a rate).

Exam Tip

You need to know the equations for net primary production and net production for the exam, so learn them both.

Tip: Net productivity is just net production expressed as a rate, i.e. per unit time.

Efficiency of energy transfer

You can use the following equation to calculate how efficient energy transfer is between one trophic level and the next:

$$\% \text{ efficiency of energy transfer} = \frac{\text{net production of trophic level}}{\text{net production of previous trophic level}} \times 100$$

Example — Maths Skills

The total energy available to the rabbits was 28 000 $\text{kJ m}^{-2} \text{ yr}^{-1}$, and their net productivity is 2000 $\text{kJ m}^{-2} \text{ yr}^{-1}$. So the percentage efficiency of energy transfer is:

$$(2000 \div 28\,000) \times 100 = \mathbf{7\% \text{ (1 s.f.)}}$$

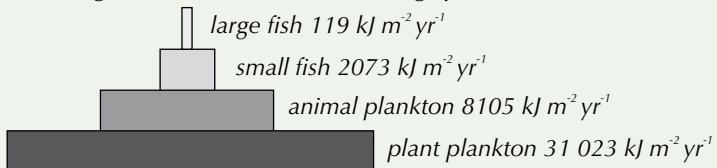
Tip: If the organisms in the previous trophic level are producers, then use 'net primary production of previous trophic level' rather than 'net production of previous trophic level'.

Tip: Increasing efficiency of energy transfer as you go up a food chain is only a general rule of thumb — there are plenty of exceptions.

As you move up a food chain (from producers to consumers) energy transfer usually becomes more efficient. For example, the efficiency of energy transfer from producer to consumer might only be 5-10%, but from consumer to consumer, it might be 15-20%. This is because plants (producers) contain more indigestible matter than animals (consumers).

Practice Questions — Application

- Q1 A scientist wanted to measure the biomass of a crop of wheat. To do so, he took a sample of the wheat and measured its dry mass.
- Describe how the scientist could have measured the dry mass of the wheat.
 - Once the scientist has a sample of dry mass, he decides to burn it in a calorimeter. What will the results from this procedure tell him about the wheat?
- Q2 The mussels in an ecosystem ingest $57\ 153\ \text{kJ m}^{-2}\ \text{yr}^{-1}$. $34\ 292\ \text{kJ m}^{-2}\ \text{yr}^{-1}$ is indigestible or lost through urine and $17\ 000\ \text{kJ m}^{-2}\ \text{yr}^{-1}$ is lost through respiration.
- Calculate the net productivity of the mussels.
The mussels provide food for crayfish which have a net productivity of $627\ \text{kJ m}^{-2}\ \text{yr}^{-1}$.
 - Calculate the efficiency of energy transfer between the mussels and the crayfish.
- Q3 The diagram below shows the net primary productivity of plant plankton in a food chain, as well as the net productivity at different trophic levels in the same food chain. Use the diagram to answer the following questions.



Tip: The two equations for net production and net primary production might get a bit confusing — just remember, if you're talking about plants (the first or primary organisms in a food chain), you need the equation for net primary production. Anything else will just be net production.

- The respiratory loss of the plant plankton is $15\ 604\ \text{kJ m}^{-2}\ \text{yr}^{-1}$. Calculate their gross primary productivity.
- The small fish ingest $5983\ \text{kJ m}^{-2}\ \text{yr}^{-1}$ and lose $2729\ \text{kJ m}^{-2}\ \text{yr}^{-1}$ in faeces and urine. Calculate the respiratory loss of the small fish.
- The respiratory loss of the large fish is $879\ \text{kJ m}^{-2}\ \text{yr}^{-1}$. Calculate the amount of energy lost in faeces and urine by the large fish.
- Give two reasons why the net productivity of the large fish is less than the net productivity of the small fish.
- Calculate the percentage efficiency of energy transfer between each stage of the food chain.

Practice Questions — Fact Recall

- What is a plant's biomass?
- What is gross primary production?
- What is meant by the term 'respiratory loss'?
- a) What is net production?
b) State the equation for net production.

2. Farming Practices and Production

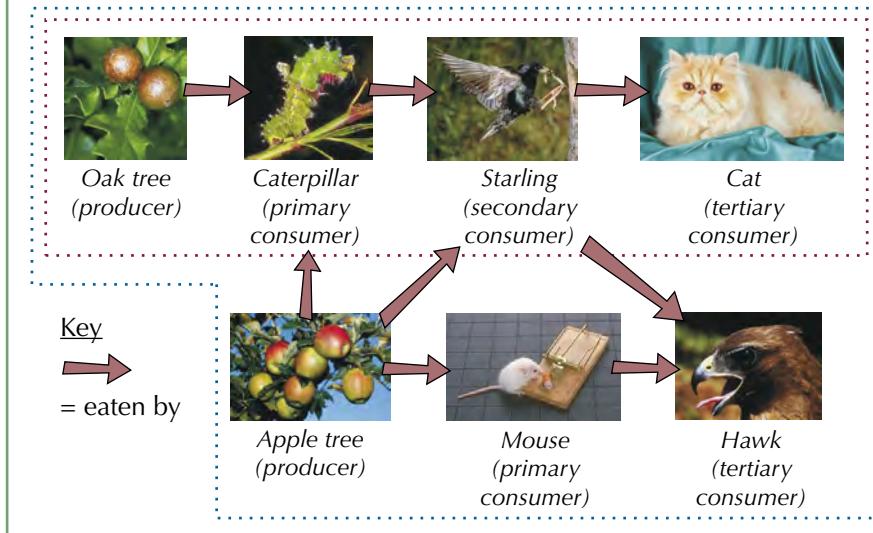
Knowing about the efficiency of energy transfer in food chains can help farmers maximise the amount of energy in the food they produce.

Food chains and food webs

Food chains and food webs show how energy is transferred through an ecosystem. Food chains show simple lines of energy transfer. Each of the stages in a food chain is called a **trophic level**. Food webs show lots of food chains in an ecosystem and how they overlap.

Example

The example below shows a food chain (red box) and a food web (blue box).



Learning Objective:

- Be able to appreciate the ways in which production is affected by farming practices designed to increase the efficiency of energy transfer by:
 - simplifying food webs to reduce energy losses to non-human food chains,
 - reducing respiratory losses within a human food chain.

Specification Reference 3.5.3

Decomposers (e.g. fungi) are also part of food webs. Decomposers break down dead or undigested material, allowing nutrients to be recycled.

Tip: There's more about decomposers on page 302.

Increasing efficiency

Most farming practices aim to increase the amount of energy that is available for human consumption. This means increasing the net primary production (NPP) of crops and the net production (NP) of livestock. There are different ways this can be done. You need to know about two of them:

- The energy lost to other organisms, e.g. pests, can be reduced through the simplification of food webs.
- The energy lost through the respiration of livestock can be reduced.

Tip: NPP is the energy in plants that's available to the next trophic level in a food chain. NP is the energy in consumers that's available to the next trophic level. See pages 296-297 for more.

Simplifying food webs

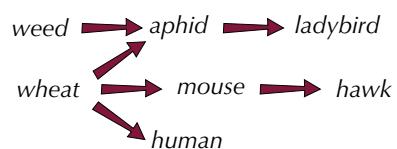
Pests are organisms that reduce the amount of energy available for crop growth and therefore the net primary production (NPP) of crops. This ultimately reduces the amount of energy available for humans. By simplifying the food web, i.e. getting rid of food chains that don't involve humans, energy losses will be reduced and the NPP of the crop will increase.

Tip: Crops are plants which are grown on a large scale for the benefit of humans, e.g. for human consumption. Livestock are animals which are bred for their produce, e.g. milk, or for human consumption.

Example

Figure 1 shows a simplified food web involving wheat — a crop plant grown for human consumption. The weed, the mouse and the aphid are pests. By eating the wheat or competing with it for energy, the pests reduce the wheat's biomass and the energy it has for further growth. This means that the wheat's NPP (and the wheat yield) is smaller — so less energy is transferred to humans. Getting rid of food chains involving the weed, the mouse and the aphid will mean that less energy is transferred to pests, increasing the efficiency of transfer to humans.

Figure 1: An example of part of a food web involving a crop.



To get rid of pests farmers need pest control. Farmers can reduce pest numbers using chemical pesticides.

Examples

- Insecticides kill insect pests that eat and damage crops. Killing insect pests means less biomass is lost from crops, so they grow to be larger, which means NPP is greater.
- Herbicides kill weeds (unwanted plant species). Killing weeds can remove direct competition with the crop for energy from the Sun. It can also remove the preferred habitat or food source of the insect pests, helping to further reduce their numbers and simplify the food web.



Figure 2: Braconid wasps lay their eggs in sphinx moth caterpillars.

Tip: Weeds also compete with the crop for water, space and nutrients.

This means that more biomass is produced and more chemical energy can be stored, increasing net production and the efficiency of energy transfer to humans. The benefits are that more food can be produced in a shorter space of time, often at lower cost. However, enhancing net production by keeping animals in pens raises ethical issues. For example, some people think that the conditions intensively reared animals are kept in cause the animals pain, distress or restricts their natural behaviour, so it shouldn't be done.

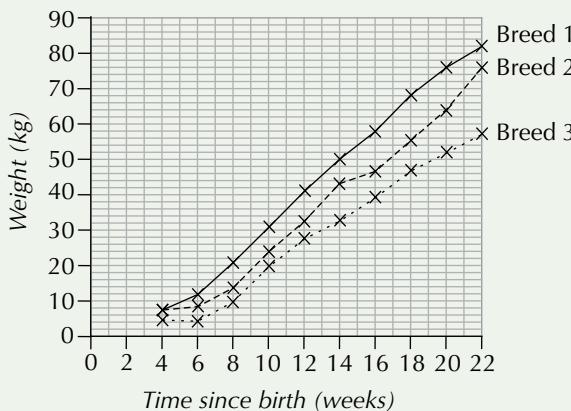


Figure 3: Chickens being kept indoors.

Practice Question — Application

- Q1 A pig farmer wants to maximise production on his farm. He collects data on three different breeds of pig as shown in the table on the right and in the graph below.

| | Meat yield (% of total body weight) |
|---------|-------------------------------------|
| Breed 1 | 64 |
| Breed 2 | 73 |
| Breed 3 | 66 |



- Calculate the rate at which breed 3 gained weight between 18 and 22 weeks.
- Which breed of pig would produce the most meat at 22 weeks? Antibiotics can be used to treat bacterial diseases in pigs.
- Suggest how using antibiotics may increase the net production of the pigs.
- Suggest and explain one other way the farmer could increase the net production of the pigs.

Tip: Remember that to find the rate you need to find the gradient of the line — see page 12.

Practice Questions — Fact Recall

- What does a food chain show?
- What does a food web show?
- Explain how simplifying a food web could increase the efficiency of energy transfer to humans.
- Give two examples of methods of pest control.

Learning Objectives:

- Know that nutrients are recycled within natural ecosystems and that the nitrogen cycle and phosphorus cycle are examples of this.
- Know that microorganisms play a vital role in recycling chemical elements such as phosphorus and nitrogen including:
 - the role of saprobionts in decomposition.
 - the role of mycorrhizae in facilitating the uptake of water and inorganic ions by plants.
 - the role of bacteria in the processes of saprobiotic nutrition, ammonification, nitrification, nitrogen fixation and denitrification.

Specification Reference 3.5.4

Tip: A symbiotic relationship is when two species live closely together and one or both species depends on the other for survival.

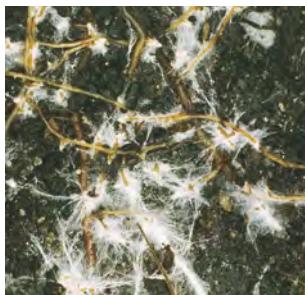


Figure 2: White hyphae of the fungus Basidiomycetes, growing on the roots of a strawberry tree.

3. Nutrient Cycles in Natural Ecosystems

A natural ecosystem is one that hasn't been changed by human activity. In natural ecosystems, nutrients such as nitrogen and phosphorus are recycled through food webs — but human activity often disrupts this.

The role of microorganisms

Microorganisms, such as bacteria and fungi, are an important part of food webs and ecosystems. Many are **saprobionts**. Saprobionts do two things:

- They feed on the remains of dead plants and animals and on their waste products (faeces and urine), breaking them down. This makes saprobionts a type of decomposer and it allows important chemical elements in the remains and waste to be recycled.
- They secrete enzymes and digest their food externally, then absorb the nutrients they need. This is known as **extracellular digestion**. During this process, organic molecules are broken down into inorganic ions.

Obtaining nutrients from dead organic matter and animal waste using extracellular digestion is known as **saprobiontic nutrition**.

Mycorrhizae

Some fungi form symbiotic relationships with the roots of plants.

These relationships are known as **mycorrhizae**. The fungi are made up of long, thin strands called hyphae, which connect to the plant's roots — see Figure 1. The hyphae greatly increase the surface area of the plant's root system, helping the plant to absorb ions from the soil that are usually scarce (e.g. phosphorus). Hyphae also increase the uptake of water by the plant. In turn, the fungi obtain organic compounds, such as glucose, from the plant.

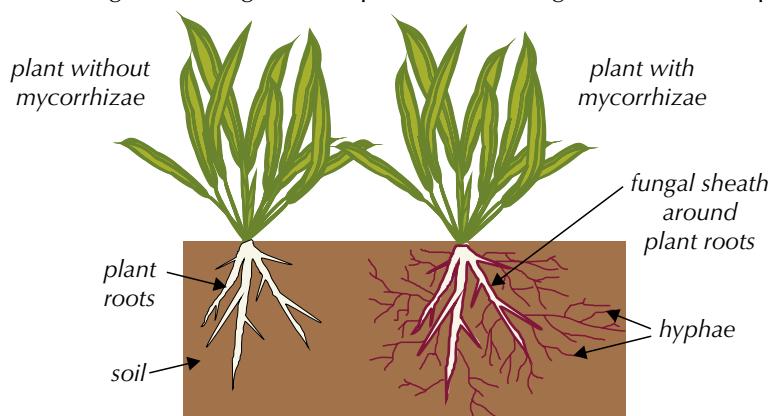


Figure 1: A plant without mycorrhizae (left) and with mycorrhizae (right).

Example

The fungus *G. intraradices* can develop mycorrhizal relationships with crops, e.g. wheat, and has been shown to increase the crop's phosphorus uptake.

The nitrogen cycle

Plants and animals need nitrogen to make proteins and nucleic acids (DNA and RNA). The atmosphere's made up of about 78% nitrogen gas, but plants and animals can't use it in that form — they need bacteria to convert it into nitrogen-containing compounds first.

The nitrogen cycle shows how nitrogen is converted into a usable form and then passed on between different living organisms and the non-living environment. It includes food chains (nitrogen is passed on when organisms are eaten), and four different processes that involve bacteria — nitrogen fixation, ammonification, nitrification and denitrification:

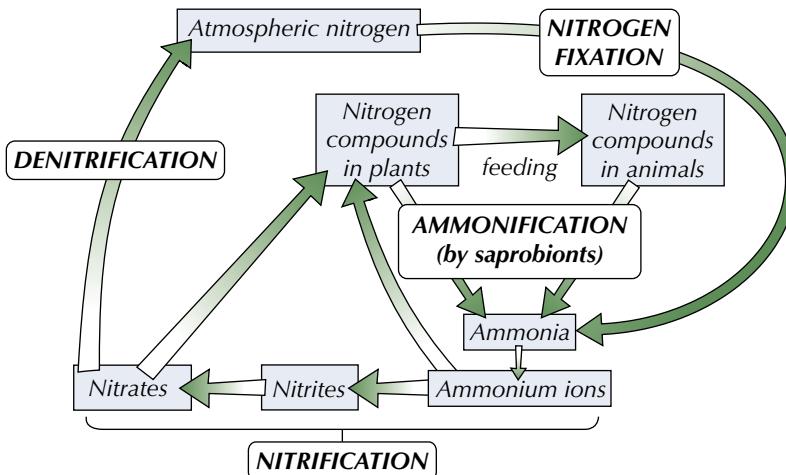


Figure 3: The four main processes in the nitrogen cycle.

Nitrogen fixation

Nitrogen fixation is when nitrogen gas in the atmosphere is turned into nitrogen-containing compounds. Biological nitrogen fixation is carried out by bacteria such as *Rhizobium*. It turns nitrogen into ammonia, which goes on to form ammonium ions in solution that can be used by plants. *Rhizobium* are found inside root nodules (growths on the roots) of leguminous plants (e.g. peas, beans and clover). They form a mutualistic relationship with the plants — they provide the plant with nitrogen compounds and the plant provides them with carbohydrates. Other nitrogen-fixing bacteria are found in the soil.

Ammonification

Ammonification is when nitrogen compounds from dead organisms are turned into ammonia by saprobionts, which goes on to form ammonium ions. Animal waste (urine and faeces) also contains nitrogen compounds. These are also turned into ammonia by saprobionts and go on to form ammonium ions.

Nitrification

Nitrification is when ammonium ions in the soil are changed into nitrogen compounds that can then be used by plants (nitrates). First nitrifying bacteria called *Nitrosomonas* change ammonium ions into nitrites. Then other nitrifying bacteria called *Nitrobacter* change nitrites into nitrates.

Denitrification

Denitrification is when nitrates in the soil are converted into nitrogen gas by denitrifying bacteria — they use nitrates in the soil to carry out respiration and produce nitrogen gas. This happens under anaerobic conditions (where there's no oxygen), e.g. in waterlogged soils.

Other ways that nitrogen gets into an ecosystem are by lightning (which fixes atmospheric nitrogen into nitrogen oxides) or by artificial fertilisers (they're produced from atmospheric nitrogen on an industrial scale in the Haber process).

Tip: Don't worry — you don't need to learn the names of the microorganisms.



Figure 4: Pink nodules of *Rhizobium* on plant roots.

Tip: A mutualistic relationship is a type of symbiotic relationship where both species benefit.

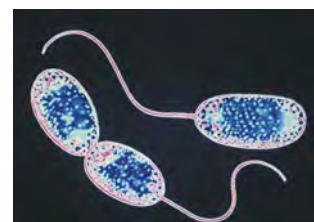


Figure 5: *Nitrobacter* bacteria.



Figure 6: *Pseudomonas aeruginosa* are a type of denitrifying bacteria.

The phosphorus cycle

Plants and animals need phosphorus to make biological molecules such as phospholipids (which make up cell membranes), DNA and ATP. Phosphorus is found in rocks and dissolved in the oceans in the form of phosphate ions (PO_4^{3-}). Phosphate ions dissolved in water in the soil can be assimilated (absorbed and then used to make more complex molecules) by plants and other producers. The phosphorus cycle shows how phosphorus is passed through an ecosystem:

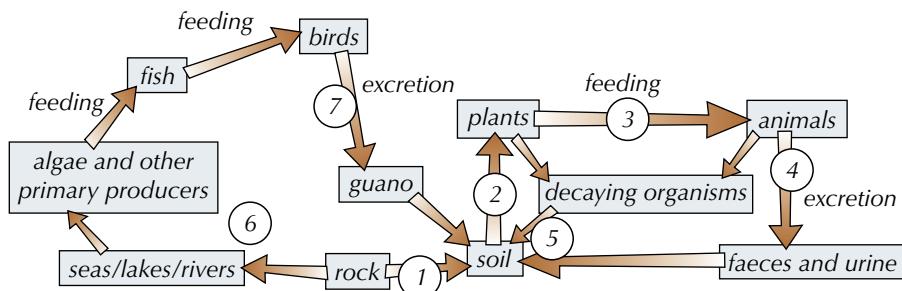


Figure 7: The phosphorus cycle.

Tip: Weathering is the breakdown of rocks by mechanical, chemical and biological processes.



Figure 8: Seabirds nesting on a cliff stained white with guano.

Tip: Tg N yr^{-1} means teragrams of nitrogen per year. 1 teragram is the same as 10^{12} grams or 1 million metric tons.

Tip: Cultivated land is land that has been used by humans to grow crops.

1. Phosphate ions in rocks are released into the soil by weathering.
2. Phosphate ions are taken into the plants through the roots. Mycorrhizae (see page 302) greatly increase the rate at which phosphorus can be assimilated.
3. Phosphate ions are transferred through the food chain as animals eat the plants and are in turn eaten by other animals.
4. Phosphate ions are lost from the animals in waste products.
5. When plants and animals die, saprobionts are involved in breaking down the organic compounds, releasing phosphate ions into the soil for assimilation by plants. These microorganisms also release the phosphate ions from urine and faeces.
6. Weathering of rocks also releases phosphate ions into seas, lakes and rivers. This is taken up by aquatic producers, such as algae, and passed along the food chain to birds.
7. The waste produced by sea birds is known as guano and contains a high proportion of phosphate ions. Guano returns a significant amount of phosphate ions to soils (particularly in coastal areas). It is often used as a natural fertiliser.

Practice Questions — Application

- Q1 The table on the right shows the rate of nitrogen fixation by various sources in the early 1990s.

| Source of nitrogen fixation | Rate of fixation (Tg N yr^{-1}) |
|-------------------------------|--|
| Lightning | 5.4 |
| Bacteria in uncultivated land | 107 |
| Bacteria in cultivated land | 31.5 |
| Fertiliser manufacture | 86 |

- a) What percentage of the total amount of nitrogen fixed by all sources was fixed by bacteria?
- b) In 1860, 120 Tg N yr⁻¹ were fixed by bacteria on uncultivated land. Calculate the overall percentage decrease in the mass of nitrogen fixed per year by bacteria on uncultivated land between 1860 and the early 1990s.
- c) Suggest why the mass of nitrogen fixed in uncultivated land decreased between 1860 and the early 1990s.
- d) Using the table, suggest one way in which the nitrogen cycle may have been altered by the action of humans.
- Q2 Heavy rain combined with poor drainage can lead to soils becoming waterlogged. Suggest and explain what might happen to the amount of nitrogen assimilated by a plant in waterlogged soil.
- Q3 Figures A and B below show simplified versions of the nitrogen and phosphorus cycles.

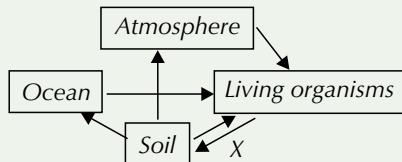


Figure A

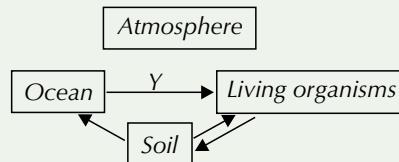


Figure B

- a) Which Figure, A or B, represents the phosphorus cycle? Give a reason for your answer.
- b) In what form is phosphorus assimilated by plants?
- c) Name the process labelled X in Figure A.
- d) Explain what is happening at the part labelled Y in Figure B.
- e) Give two ways in which phosphate ions are returned from living organisms to the soil.

Exam Tip

Make sure you know your way around basic maths — you'll be asked some maths questions in the exams, and simple calculations such as percentage decrease are easy marks if you know what you're doing.

Practice Questions — Fact Recall

- Q1 a) Name two types of saprobiont.
b) How do saprobionts digest their food?
- Q2 a) What are mycorrhizae?
b) Explain how mycorrhizae increase water and mineral ion uptake by plants.
- Q3 Name the four main processes in the nitrogen cycle.
- Q4 Describe the role of saprobionts in the phosphorus cycle.
- Q5 a) What is guano?
b) Why is guano important in the phosphorus cycle?

Learning Objectives:

- Know that natural and artificial fertilisers can be used to replace the nitrates and phosphates lost by harvesting plants and removing livestock.
- Understand the environmental issues arising from the use of fertilisers including leaching and eutrophication.

Specification Reference 3.5.4



Figure 1: Pellets of a chemical fertiliser — diammonium phosphate.



Figure 2: A tractor spreading natural fertiliser — liquid manure.

4. Fertilisers and Eutrophication

Farmers can add extra nutrients, e.g. nitrogen and phosphorus, to the soil to increase the productivity of crops, but this can cause environmental problems. It's a delicate balance between adding too much and too little.

Loss of nutrients

Crops take in minerals from the soil as they grow and use them to build their own tissues. When crops are harvested, they're removed from the field where they're grown rather than being allowed to die and decompose there. This means the mineral ions that they contain (e.g. phosphates and nitrates) are not returned to the soil by decomposers in the nitrogen or phosphorus cycles.

Phosphates and nitrates are also lost from the system when animals or animal products are removed from the land. Animals eat grass and other plants, taking in their nutrients. When they are taken elsewhere for slaughter or transferred to a different field, the nutrients aren't replaced through their remains or waste products.

Using fertilisers

Adding fertiliser replaces the lost minerals, so more energy from the ecosystem can be used for growth, increasing the efficiency of energy transfer. Fertilisers can be artificial or natural.

- Artificial fertilisers are inorganic — they contain pure chemicals (e.g. ammonium nitrate) as powders or pellets.
- Natural fertilisers are organic matter — they include manure, composted vegetables, crop residues (the parts left over after the harvest) and sewage sludge.

Environmental issues

Sometimes more fertiliser is applied than the plants need or are able to use at a particular time. This can lead to the fertilisers **leaching** into waterways. Leaching is when water-soluble compounds in the soil are washed away, e.g. by rain or irrigation systems. They're often washed into nearby ponds and rivers. This can lead to **eutrophication** (see next page).

Inorganic ions in chemical fertilisers are relatively soluble. This means that excess minerals that are not used immediately are more likely to leach into waterways. Leaching is also more likely to occur if the fertiliser is applied just before heavy rainfall.

Leaching is less likely with natural fertilisers — that's because the nitrogen and phosphorus are still contained in organic molecules that need to be decomposed by microorganisms before they can be absorbed by plants. This means that their release into the soil for uptake by plants is more controlled. The leaching of phosphates is less likely than the leaching of nitrates because phosphates are less soluble in water.

Using fertilisers may also change the balance of nutrients in the soil — too much of a particular nutrient can cause crops and other plants to die.

Eutrophication

Eutrophication is caused by excess nutrients. The process of eutrophication is explained below and illustrated in Figure 3:

1. Mineral ions leached from fertilised fields stimulate the rapid growth of algae in ponds and rivers.
2. Large amounts of algae block light from reaching the plants below.
3. Eventually the plants die because they're unable to photosynthesise enough.
4. Bacteria feed on the dead plant matter. The increased numbers of bacteria reduce the oxygen concentration in the water by carrying out aerobic respiration.
5. Fish and other aquatic organisms die because there isn't enough dissolved oxygen.

Tip: Eutrophication is where too many mineral ions in the water cause a sequence of "mega-growth, mega-death and mega-decay" involving most of the plant and animal life in the water.

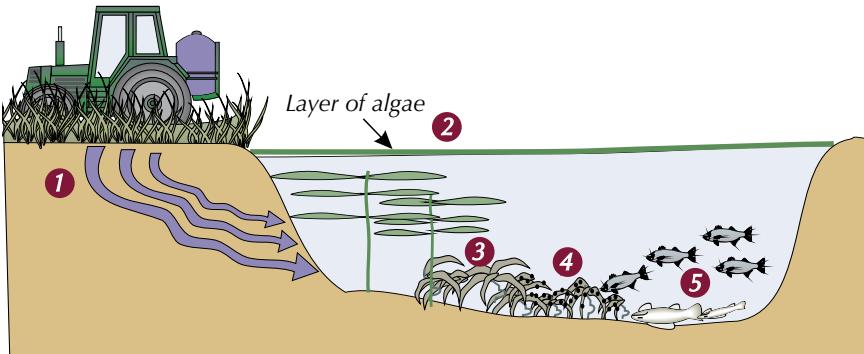


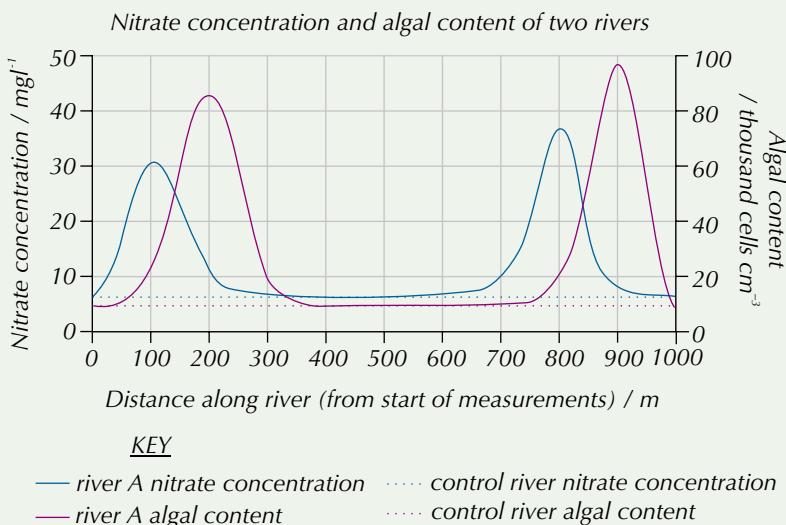
Figure 3: The process of eutrophication.



Figure 4: Algae growing on the Thames estuary.

Practice Questions — Application

A study was conducted to investigate the changes in nitrate concentration and algal content down a stretch of river with two farms along it (river A). In the same study, the nitrate concentration and algal content of a river without farmland along it (control river) was also recorded. The results are shown in the graph below:



Tip: The questions about this graph are on the next page.

Tip: There's more on drawing conclusions on page 15.

- Q1 a) Suggest the distance at which the two farms are situated along river A. Explain your answer.
- b) i) Calculate the percentage increase in peak nitrate concentration between the first and second farm along the river.
- ii) Suggest a reason for this increase.
- Q2 What do the results of the control river show?
- Q3 a) Draw a conclusion from the data shown in the graph.
- b) Suggest an explanation for your conclusion.
- Q4 Describe and explain the possible consequences of the peaks in the algal content of river A at 200 m and 900 m.

Practice Questions — Fact Recall

- Q1 Explain how mineral ions are lost from crop fields.
- Q2 Give an example of a natural fertiliser.
- Q3 By what process do fertilisers get into waterways?
- Q4 Name the process that can occur as a result of excess fertilisers in waterways.

Section Summary

Make sure you know...

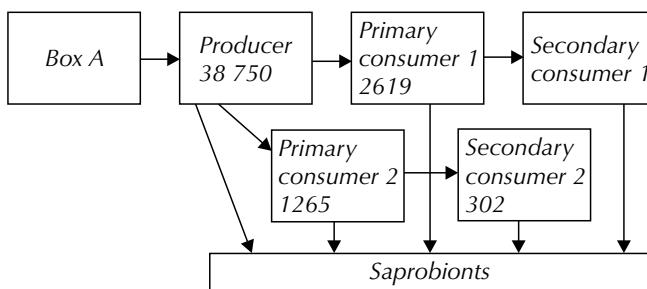
- That plants make organic compounds during photosynthesis using light energy from the Sun and carbon dioxide from the atmosphere (in land-based ecosystems) and dissolved in water (in aquatic ecosystems).
- That some of the sugars produced during photosynthesis are used immediately for respiration.
- That the rest of the sugars are used to make biological molecules — these form the plants' biomass.
- That a plant's biomass is the mass of living material or the chemical energy that is stored in the plant.
- That biomass can be measured in terms of the mass of carbon that an organism contains or the dry mass of its tissue per unit area.
- That dry mass is the mass of an organism with the water removed and that the mass of carbon is approximately 50% of the dry mass.
- That the chemical energy stored in an organism can be estimated using a calorimeter.
- That the gross primary production (*GPP*) is the total amount of chemical energy that is converted from light energy by plants during photosynthesis, in a given area.
- That the energy lost to the environment as heat when plants respire is called the respiratory loss (*R*).
- That the net primary production (*NPP*) is the energy remaining from *GPP* after respiratory loss and that this is shown by the formula $NPP = GPP - R$.
- That when primary production is expressed as a rate it is called primary productivity.
- That *NPP* is the energy available for a plant's growth and reproduction and is also the energy available to the next trophic level.
- That consumers also store energy as biomass.

- That about 90% of the energy available to consumers is lost to the environment — some is not ingested (e.g. roots, bones), some is indigestible and is egested as faeces, and some is lost through respiration or the excretion of urine.
- That the remaining energy or net production (N) of consumers can be calculated using the following formula: $N = I - (F + R)$, where I is the chemical energy in ingested food, F is the chemical energy lost in faeces and urine and R is the energy lost through respiration.
- That when net production of consumers is expressed as a rate it can be called net or secondary productivity.
- That food chains and webs show how energy is transferred through an ecosystem.
- That farming practices try to maximise production by simplifying food webs to reduce energy losses (e.g. by removing pests using pesticides or biological agents) and by reducing respiratory losses (e.g. by limiting the movement of livestock and keeping livestock warm).
- That microorganisms such as bacteria and fungi are saprobionts and recycle nutrients through food webs through the decomposition of dead plants and animals and waste matter.
- That some fungi form symbiotic relationships with plants known as mycorrhizae, and that these increase the rate of water and mineral ion uptake by plants by increasing the surface area of the plants' root system.
- The role of microorganisms in the four main processes of the nitrogen cycle: nitrogen fixation, ammonification, nitrification and denitrification, including the role of saprobionts.
- How phosphorus is recycled in natural ecosystems, including the role of mycorrhizae.
- That natural and artificial fertilisers are used to replace the nitrates and phosphates that are lost from the soil when crops are harvested or livestock are removed from the area.
- That leaching is when water soluble compounds in the soil are washed away, e.g. by rain.
- That the leaching of fertilisers can cause eutrophication.
- That eutrophication involves the rapid growth of algae, causing plants to die from lack of light, and that bacteria feeding on the dead organic matter decrease the oxygen concentration causing other aquatic organisms to die as well.

Exam-style Questions

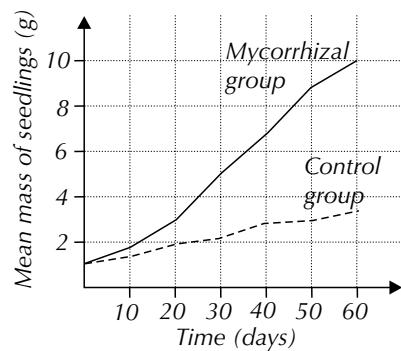
- 1 **Figure 1** shows the net productivity of some organisms in a food web. All the figures are in $\text{kJ m}^{-2} \text{yr}^{-1}$.

Figure 1



- 1.1 Name the source of energy represented by Box A and the process by which this energy is transferred to the producer. (2 marks)
- 1.2 The respiratory loss of secondary consumer 1 is $785 \text{ kJ m}^{-2} \text{yr}^{-1}$ and the energy lost through urine and faeces is $1571 \text{ kJ m}^{-2} \text{yr}^{-1}$. Calculate the net productivity of secondary consumer 1. (1 mark)
- 1.3 Calculate the difference in the percentage efficiency of energy transfer between the producer and primary consumer 1, and the producer and primary consumer 2. (2 marks)
- 1.4 Describe the role of the saprobionts in this food web. (2 marks)
- 2 A group of scientists were investigating the effect of mycorrhizae on the growth of plant seedlings. The scientists inoculated a group of seedlings with a mycorrhizal culture and grew them under controlled conditions for 60 days. A control group of seedlings was not inoculated with the mycorrhizal culture but was grown under the same conditions. The seedlings in each set were periodically weighed and their mean mass recorded. The results are shown in **Figure 2**.
- 2.1 Explain what is meant by the term 'mycorrhizae'. (1 mark)

Figure 2



- 2.2** Suggest **two** environmental conditions under which the seedlings were grown, which should have been controlled by the scientists. (1 mark)
- 2.3** Calculate the average rate of growth in the mycorrhizal group between 30 and 60 days. Give your answer in g day⁻¹. (1 mark)
- 2.4** Suggest an explanation for the differences between the mycorrhizal group and the control group shown in **Figure 2**. (3 marks)

- 3** A study was carried out to investigate the effect of different types of pest control on greenfly — a pest species.

Three fields of potato crops, each with a greenfly infestation, were treated with a different type of pest control — one with a pesticide, the other with lacewing insects (a natural predator of greenfly) and the third with an integrated system that made use of both a pesticide and lacewings.

The study included a negative control. The number of greenfly in each field was recorded over time. The net primary production of each field was also measured. The graphs below show the results.

Figure 3

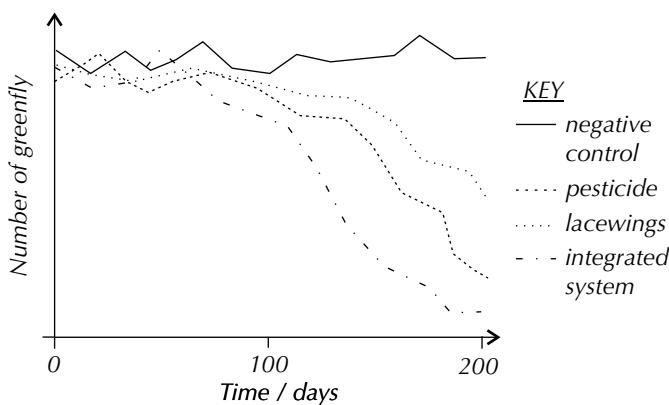
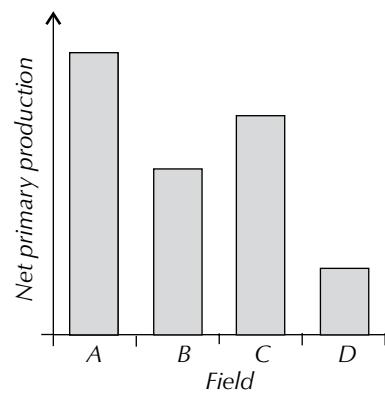


Figure 4



- 3.1** Suggest what the negative control would have been in this study. (1 mark)
- 3.2** Using the results of **Figure 3** and your own knowledge, identify which field in **Figure 4** is the negative control field and which is the field treated with the integrated system. Explain your answers. (4 marks)
- Net productivity can be thought of as the rate at which energy is stored or the rate at which biomass is added.
- 3.3** Suggest and explain a method for determining the rate at which biomass is added by a potato crop over a study period. (4 marks)

Topic 6

A: Stimuli and Responses

Learning Objectives:

- Know that organisms increase their chance of survival by responding to changes in their environment.
- Know that taxes and kineses are simple responses that maintain a mobile organism in a favourable environment.
- Be able to investigate the effect of an environmental variable on the movement of an animal using either a choice chamber or a maze (Required Practical 10).

Specification Reference 3.6.1.1

Tip: If an organism moves towards a stimulus it's a positive taxis, and if it moves away from a stimulus it's a negative taxis.
So in the first example, woodlice show a negative taxis to light.

Tip: Taxes is the plural of taxis, kineses is the plural of kinesis.

Tip: The word before 'taxis' tells you what the organism is responding to, e.g. phototaxis is a response to light.

1. Survival and Response

In order to survive, organisms need to respond to what's going on around them. Otherwise they'd find themselves in a pretty unfavourable position...

Responding to the environment

Organisms increase their chances of survival by responding to changes in their external environment. Animals and plants respond in different ways.

Examples

- Animals can move away from harmful environments such as places that are too hot or too cold.
- Plants can't actually move themselves, but they can change the way they grow in an attempt to find more favourable environmental conditions. E.g. seedlings growing in dark conditions can rapidly develop very long, thin stems to increase their chances of finding light.

Organisms also respond to changes in their internal environment to make sure that the conditions are always optimal for their metabolism (all the chemical reactions that go on inside them).

Any change in the internal or external environment, e.g. a change in temperature, light intensity or pressure, is called a **stimulus**.

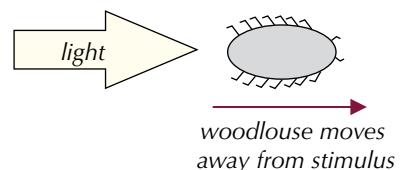
Simple responses

Simple mobile organisms, e.g. woodlice, have simple responses to keep them in a favourable environment. Their response can either be tactic or kinetic:

Tactic response (taxis) — directional movement in response to a stimulus.
The direction of the stimulus affects the response.

Example

Woodlice show a tactic response to light — they move away from a light source. This helps them survive as it keeps them concealed under stones during the day (where they're safe from predators) and keeps them in damp conditions (which reduces water loss).



woodlouse moves away from stimulus

Kinetic response (kinesis) — non-directional (random) movement in response to a stimulus. The intensity of the stimulus affects the response.

Example

Woodlice show a kinetic response to humidity. In high humidity they move slowly and turn less often, so that they stay where they are. As the air gets drier, they move faster and turn more often, so that they move into a new area. This response helps woodlice move from drier air to more humid air, and then stay put. This improves their chances of survival — it reduces their water loss and it helps to keep them concealed.

Investigating simple animal responses

A choice chamber is a container with different compartments, in which you can create different environmental conditions. It can be used to investigate how animals, such as woodlice or maggots, respond to conditions like light intensity or humidity in the laboratory. Here's how you can use a choice chamber:

REQUIRED PRACTICAL 10

1. Construct a choice chamber using the equipment shown in Figure 1.

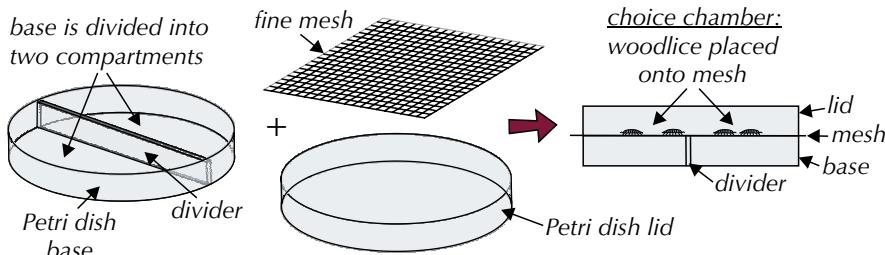


Figure 1: How to construct a choice chamber.

2. To investigate the effect of light intensity on woodlouse movement, cover one half of the lid (including the sides) with black paper. This will make one side of the chamber dark. Put damp filter paper in both sides of the base to make the humidity constant throughout the chamber.
3. Place 10 woodlice on the mesh in the centre of the chamber and position the lid on the mesh so it's lined up with the base below.
4. After 10 minutes, take off the lid and record the number of woodlice on each side of the chamber. Try to minimise the amount of time the lid is off, so that the environmental conditions created aren't disturbed.
5. Repeat the experiment after gently moving the woodlice back to the centre. You can use a small, soft paintbrush to help with moving the woodlice if necessary. You should find that most woodlice end up on the dark side of the choice chamber (a tactic response to light).
6. To investigate humidity, place some damp filter paper in one side of the base and a desiccating (drying) agent (such as anhydrous calcium chloride) in the other side. Don't cover the lid with paper. Put the lid on and leave the chamber for 10 minutes for the environmental conditions to stabilise before carrying out steps 3-5 above.

You can also investigate simple animal responses using a maze. For example, a paper maze can be used to investigate turning behaviour in woodlice and whether it's affected by light intensity.

Tip: Simple responses are automatic responses to a stimulus — the organism doesn't 'choose' where to move.

Tip: Don't forget to assess all the risks involved before you begin your experiment.

Tip: For ethical reasons, you should handle the woodlice carefully and return them to their natural habitat as soon as possible. Make sure you wash your hands after handling the woodlice.

Tip: Be careful if you use anhydrous calcium chloride. It's an irritant to the eyes and skin and generates heat on contact with water. Make sure you're wearing eye protection.

Tip: After being forced to turn in a particular direction, woodlice will often turn in the opposite direction next time they have a free choice. This turn alternation can increase the chances of them finding more favourable conditions because it means that they move into different areas.

Practice Question — Application

- Q1 *E. coli*, a type of bacteria, have been observed to move towards the highest concentration of oxygen in their surroundings.
- a) What type of simple response is this?
 - b) Suggest why *E. coli* move in this way.

Practice Questions — Fact Recall

- Q1 Why is it important that organisms respond to stimuli?
- Q2 What is a tactic response?
- Q3 What is a kinetic response?

Learning Objective:

- Know that a simple reflex has a protective effect, exemplified by a three-neurone simple reflex.

Specification Reference 3.6.1.1

2. Nervous Communication

In order to respond to changes in the environment, an organism needs to pass information between different areas of its body. In animals some of this communication is carried out using nerve impulses.

Receptors and effectors

Receptors detect stimuli — they can be cells, or proteins on cell surface membranes. There are loads of different types of receptors that detect different stimuli, e.g. baroreceptors are a type of receptor that detect changes in blood pressure, but receptors are specific to one type of stimulus (see page 320).

Effectors are cells that bring about a response to a stimulus, to produce an effect. Effectors include muscle cells and cells found in glands, e.g. the pancreas. Receptors communicate with effectors via the nervous system or the hormonal system, or sometimes using both.

The nervous system

Neurones (nerve cells)

The nervous system is made up of a complex network of cells called neurones. There are three main types of neurone:

- Sensory neurones** transmit electrical impulses from receptors to the central nervous system (CNS) — the brain and spinal cord.
- Motor neurones** transmit electrical impulses from the CNS to effectors.
- Relay neurones** (also called intermediate neurones, interneurons or association neurones) transmit electrical impulses between sensory neurones and motor neurones.

Nervous communication

A stimulus is detected by receptor cells and an electrical impulse is sent along a sensory neurone. When an electrical impulse reaches the end of a neurone chemicals called **neurotransmitters** take the information across the gap (called a synapse) to the next neurone, where another electrical impulse is generated (see p. 335). The CNS (the coordinator) processes the information and sends impulses along motor neurones to an effector (see Figure 1).



Figure 1: The pathway of nervous communication.

Example

A real-life example of nervous communication is when you see a friend waving to you and you wave back in response:

- Stimulus** — you see a friend waving.
- Receptors** — light receptors (photoreceptors) in your eyes detect the wave. The electrical impulse is carried by a sensory neurone to the CNS.
- CNS** — processes information and sends an electrical impulse along a motor neurone.
- Effectors** — muscle cells are stimulated by the motor neurone.
- Response** — muscles contract to make your arm wave.

The nervous response

When an electrical impulse reaches the end of a neurone, chemical messengers called neurotransmitters are secreted directly onto cells (e.g. muscle cells) — so the nervous response is localised. Neurotransmitters are quickly removed once they've done their job, so the response is short-lived. Electrical impulses are really fast, so the response is usually rapid — this allows animals to react quickly to stimuli.

Tip: The cells that neurotransmitters are released onto are called target cells — they have specific receptors for the neurotransmitters (see page 335 for more).

Simple reflexes

A simple reflex is a rapid, involuntary response to a stimulus. The pathway of communication goes through the spinal cord but not through conscious parts of the brain, so the response happens automatically. Because you don't have to spend time deciding how to respond, information travels really fast from receptors to effectors.

Simple reflexes are protective — they help organisms to avoid damage to the body because the response happens so quickly.

Tip: Nervous impulses that involve the conscious brain are voluntary responses — you have to think about them. Reflexes don't involve the conscious brain so they're involuntary responses — your body responds without thinking about it first.

The reflex arc

The pathway of neurones linking receptors to effectors in a simple reflex is called a reflex arc. Three neurones are involved — a sensory neurone, a relay neurone and a motor neurone (see Figure 2).

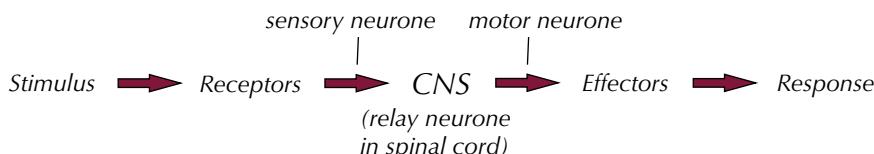
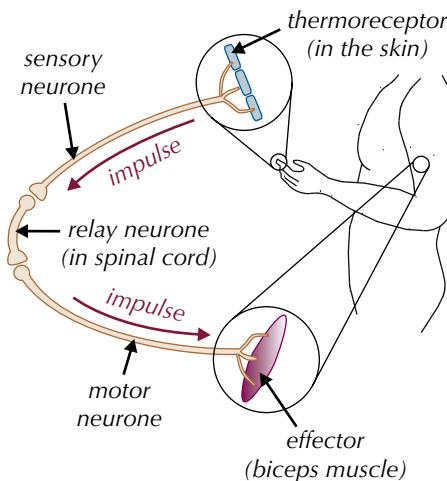


Figure 2: The pathway of nervous communication in a simple reflex arc.

Example

A real-life example of a simple reflex is the hand-withdrawal response to heat:

- **Stimulus** — you touch a hot surface.
- **Receptors** — thermoreceptors (heat receptors) in your skin detect the heat stimulus. A sensory neurone carries the impulse to the CNS.
- **CNS** — a relay neurone in your spinal cord carries the impulse to a motor neurone.
- **Effectors** — the motor neurone carries the impulse to muscle cells in your biceps.
- **Response** — your biceps muscle contracts to pull your hand away from the heat source and stop your hand from being damaged.



Exam Tip

You need to learn a simple reflex arc involving three neurones for your exam. The hand-withdrawal response to heat is a really common example.

If there's a relay neurone involved in the simple reflex arc then it's possible to override the reflex, e.g. in the example above your brain could tell your hand to withstand the heat.



Figure 3: A doctor testing the knee-jerk reflex of a patient.

Tip: When you're asked to 'suggest' answers, you're not expected to be able to give an exact, detailed answer. Instead, you're expected to use your knowledge to make educated and plausible suggestions.

Practice Questions — Application

- Q1 The knee-jerk reflex involves lightly tapping a person on the patellar tendon (just below the knee-cap) with a tendon hammer. When this happens, the quadriceps muscle (in the thigh) immediately contracts, causing the person's lower leg to jerk forward.
- This response is a reflex. Suggest one way in which the response would differ if it was not a reflex.
 - Name the stimulus and the effector in the knee-jerk reflex.
 - The knee-jerk reflex is unusual because the sensory neurone synapses directly onto the motor neurone in the spinal cord.
 - Describe how this differs from a simple reflex, such as the hand-withdrawal response to heat.
 - Suggest what effect tapping the patellar tendon might have in someone with a spinal cord injury. Explain your answer.
- Q2 Many nociceptors (pain receptors) are located in the skin.
- Describe the pathway of nervous communication that would take place in a healthy person if they pricked their finger with a pin.
- Congenital insensitivity to pain is a condition where the body does not feel physical pain. The condition is a result of non-functional nociceptors. The ability of sufferers to feel a light touch is usually normal.
- Suggest why people with this condition are able to feel a light touch even though they're unable to feel pain.
 - Suggest why it's beneficial to an organism to be able to detect and respond to pain.

Practice Questions — Fact Recall

- Q1 What is the role of a receptor?
- Q2 Give two types of cell that act as effectors.
- Q3 Describe the roles of the following types of neurone:
 - sensory,
 - motor,
 - relay.
- Q4 a) Describe the pathway of nervous communication from stimulus to response, in a voluntary response.
b) Which part of the pathway acts as the coordinator in this response?
- Q5 Explain why nervous communication leads to a localised and short-lived response.
- Q6 Reflexes are involuntary responses to stimuli. Explain why they are involuntary.
- Q7 Why do simple reflexes help an organism to avoid damage to their body?

3. Responses in Plants

Just like animals, plants also respond to stimuli. Not surprisingly they use a different system to animals — it's all about tropisms and growth factors in the plant world...

Tropisms

Flowering plants, like animals, increase their chances of survival by responding to changes in their environment.

Examples

- They sense the direction of light and grow towards it to maximise light absorption for photosynthesis.
- They can sense gravity, so their roots and shoots grow in the right direction.
- Climbing plants have a sense of touch, so they can find things to climb and reach the sunlight.

A tropism is the response of a plant to a directional stimulus (a stimulus coming from a particular direction). Plants respond to stimuli by regulating their growth. A positive tropism is growth towards the stimulus, whereas a negative tropism is growth away from the stimulus.

Phototropism

Phototropism is the growth of a plant in response to light. Shoots are positively phototropic and grow towards light (see Figure 1). Roots are negatively phototropic and grow away from light (see Figure 2).

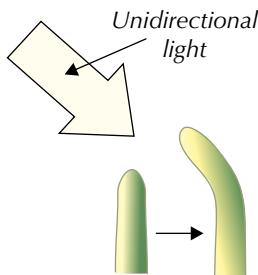


Figure 1: Phototropism in shoots.

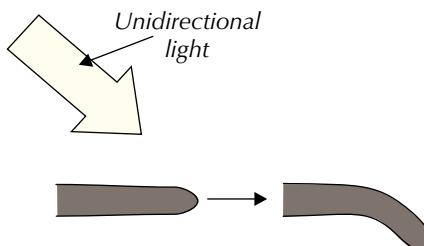


Figure 2: Phototropism in roots.

Gravitropism

Gravitropism is the growth of a plant in response to gravity. Shoots are negatively gravitropic and grow upwards (see Figure 4). Roots are positively gravitropic and grow downwards (see Figure 5).

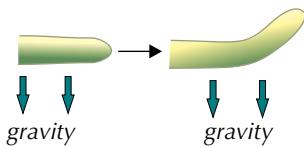


Figure 4: Gravitropism in shoots.

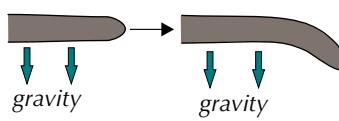


Figure 5: Gravitropism in roots.

Auxins

Plants respond to directional stimuli using specific growth factors — these are hormone-like chemicals that speed up or slow down plant growth. Plant growth factors are produced in the growing regions of the plant (e.g. shoot and root tips) and they move to where they're needed in the other parts of the plant.

Learning Objectives:

- Know that in flowering plants, specific growth factors move from growing regions to other tissues, where they regulate growth in response to directional stimuli.
- Know the effect of different concentrations of indoleacetic acid (IAA) on cell elongation in the roots and shoots of flowering plants as an explanation of gravitropism and phototropism in flowering plants.

Specification Reference 3.6.1.1



Figure 3: A radish seedling showing positive phototropism.

Tip: Gravitropism is sometimes referred to as geotropism.



Figure 6: A radish seedling showing negative gravitropism.

Tip: There are other classes of growth factors that affect growth in different ways, e.g. a growth factor called gibberellin stimulates flowering and seed germination.

Tip: Phloem is a tissue which transports sugars around a plant.

Exam Tip
You need to learn these examples for the exam.

Tip: Remember, root growth is inhibited by high concentrations of IAA. The opposite is true in shoots — high concentrations of IAA promote shoot growth.

Tip: Remember, gravitropism is the growth of a plant in response to gravity.

Growth factors called **auxins** are produced in the tips of shoots and diffuse backwards to stimulate the cell just behind the tips to elongate — this is where cell walls become loose and stretchy, so the cells get longer (see Figure 7). If the tip of a shoot is removed, no auxin will be available and the shoot stops growing.

Auxins stimulate growth in shoots but high concentrations inhibit growth in roots.

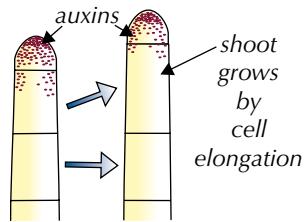


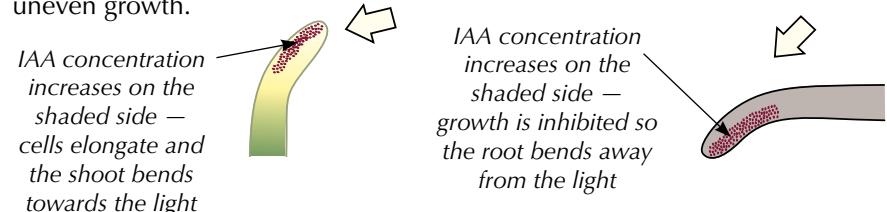
Figure 7: Effect of auxins on shoot growth.

Indoleacetic acid (IAA)

Indoleacetic acid (IAA) is an important auxin that's produced in the tips of shoots and roots in flowering plants. It's moved around the plant to control tropisms — it moves by diffusion and active transport over short distances, and via the phloem over long distances. This results in different parts of the plant having different concentrations of IAA. The uneven distribution of IAA means there's uneven growth of the plant.

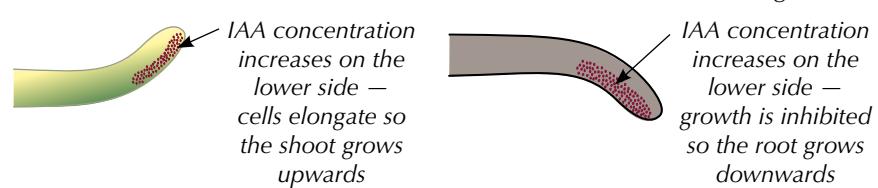
Example — phototropism

IAA moves to the more shaded parts of the shoots and roots, so there's uneven growth.



Example — gravitropism

IAA moves to the underside of shoots and roots, so there's uneven growth.



Interpreting experimental data about IAA

In the exam, you could be given some experimental data on IAA and then be asked to interpret the data. You could get something that looks a little like this:

Example

An experiment was carried out to investigate the role of IAA in shoot growth. Eight shoots, equal in height and mass, had their tips removed. Sponges soaked in glucose and either IAA or water were then placed where the tip should be. Four shoots were then placed in the dark (experiment A) and the other four shoots were exposed to a light source, directed at them from the right (experiment B) — see Figure 8 (next page).

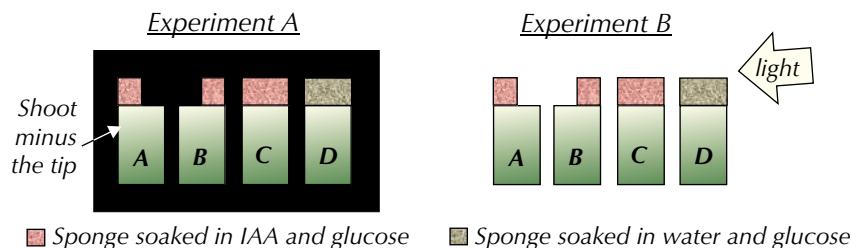


Figure 8: An investigation into the role of IAA.

After two days the amount of growth (in mm) and direction of growth was recorded. The results are shown in the table on the right.

| | Growth / mm | | | |
|----------------------|-------------|----------|-------------|-------------|
| | Shoot A | Shoot B | Shoot C | Shoot D |
| Experiment A (dark) | 6, right | 6, left | 6, straight | 1, straight |
| Experiment B (light) | 8, right | 8, right | 8, right | 3, straight |

You could be asked to explain the data...

The results show how the movement of IAA controls phototropism in plant shoots. In experiment A shoot A, the IAA diffused straight down from the sponge into the left-hand side of the shoot. This stimulated the cells on this side to elongate, so the shoot grew towards the right. In shoot B, the opposite occurred, making the shoot grow towards the left. In shoot C, equal amounts of IAA diffused down both sides, making all the cells elongate at the same rate.

In experiment B, the shoots were exposed to a light source. The IAA diffused into the shoot and accumulated on the shaded side (left-hand side) regardless of where the sponge was placed. Shoots A, B and C all grew towards the right because most IAA accumulated on the left, stimulating cell elongation there.

Tip: Remember, growth factors are produced in shoot tips. So by removing the tips, any IAA already present is removed.

Exam Tip

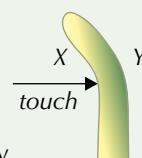
You might be asked about experimental design in the exam. A good thing to think about is the use of controls. In this experiment the negative control treatment was the sponge soaked in water (and glucose) which was included to show that it was the IAA causing the observed effects and nothing else.

Tip: All sponges used were soaked in glucose to provide energy for growth of shoots. Photosynthesis can't take place in the dark so the growth of seedlings in experiment A might have been limited if they weren't provided with glucose (an external energy source).

Practice Question — Application

Q1 Thigmotropism is a plant growth response to touch.

- In the diagram on the right, does the shoot display positive or negative thigmotropism?
- Is the concentration of auxins, such as IAA, likely to be highest at the point labelled X or Y? Explain why.



Practice Questions — Fact Recall

Q1 What name is given to the growth of a plant in response to light?

Q2 Plant shoots are negatively gravitropic. What does this mean?

Q3 What parts of a plant produce growth factors?

Q4 How do auxins affect plant growth?

Q5 What is indoleacetic acid (IAA) and where is it produced?

Q6 How does IAA move around a plant?

Q7 Explain how the distribution of IAA affects the growth of:

- shoots in response to light.
- roots in response to gravity.

Learning Objectives:

- Know that the Pacinian corpuscle is an example of a receptor and illustrates that:
 - receptors only respond to specific stimuli.
 - stimulation of a receptor leads to the establishment of a generator potential.
- Know that deformation of stretch-mediated sodium ion channels in a Pacinian corpuscle leads to the establishment of a generator potential.
- Know the basic structure of a Pacinian corpuscle.
- Understand the human retina in sufficient detail to show how differences in sensitivity to light, sensitivity to colour and visual acuity are explained by differences in the optical pigments of rods and cones and the connections rods and cones make in the optic nerve.

Specification Reference 3.6.1.2

Tip: Potential difference across a cell membrane is usually measured in millivolts (mV).

Tip: There's much more on action potentials on pages 331-333.

4. Receptors

Receptors detect stimuli. They pass information about stimuli along the nervous pathway... and you need to know how they work.

How receptors work

Receptors are specific — they only detect one particular stimulus, e.g. light or glucose concentration or pressure (e.g. Pacinian corpuscles — see next page). There are many different types of receptor that each detect a different type of stimulus. Some receptors are cells, e.g. photoreceptors are receptor cells that connect to the nervous system (see p. 322). Some receptors are proteins on cell surface membranes, e.g. glucose receptors are proteins found in the cell membranes of some pancreatic cells.

Receptors in the nervous system convert the energy of the stimulus into the electrical energy used by neurones. Here's how they work...

The resting potential

When a nervous system receptor is in its resting state (not being stimulated), there's a difference in charge between the inside and the outside of the cell — the inside is negatively charged relative to the outside (see Figure 1). This means there's a **voltage** across the membrane. Voltage is also known as **potential difference**. The potential difference when a cell is at rest is called its **resting potential**. The resting potential is generated by ion pumps and ion channels (see p. 330).

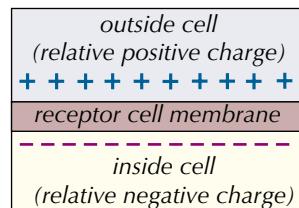


Figure 1: Relative charges either side of a receptor cell membrane at rest.

The generator potential

When a stimulus is detected, the cell membrane is excited and becomes more permeable, allowing more ions to move in and out of the cell — altering the potential difference. The change in potential difference due to a stimulus is called the **generator potential**. A bigger stimulus excites the membrane more, causing a bigger movement of ions and a bigger change in potential difference — so a bigger generator potential is produced (see Figure 2).

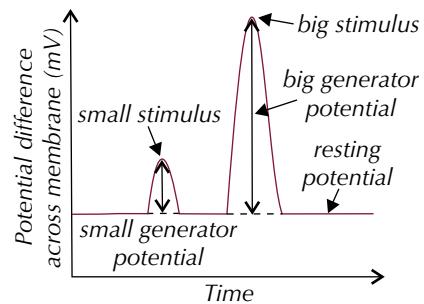


Figure 2: The bigger stimulus produces the bigger generator potential.

The action potential

If the generator potential is big enough it'll trigger an action potential — an electrical impulse along a neurone. An action potential is only triggered if the generator potential reaches a certain level called the **threshold level**. Action potentials are all one size, so the strength of the stimulus is measured by the frequency of action potentials (the number of action potentials triggered during a certain time period). If the stimulus is too weak the generator potential won't reach the threshold, so there's no action potential (Figure 3).

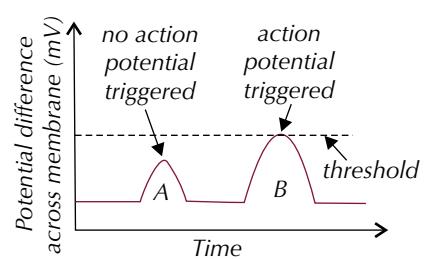


Figure 3: Generator potential not reaching the threshold (A) and reaching the threshold (B).

Pacinian corpuscles

Pacinian corpuscles are mechanoreceptors — they detect mechanical stimuli, e.g. pressure and vibrations. They're found in your skin. Pacinian corpuscles contain the end of a sensory neurone, imaginatively called a sensory nerve ending. The sensory nerve ending is wrapped in loads of layers of connective tissue called lamellae (see Figure 4).

When a Pacinian corpuscle is stimulated, e.g. by a tap on the arm, the lamellae are deformed and press on the sensory nerve ending. This causes the sensory neurone's cell membrane to stretch, deforming the **stretch-mediated sodium ion channels**. The channels open and sodium ions diffuse into the cell, creating a generator potential. If the generator potential reaches the threshold, it triggers an action potential.

Tip: Pacinian corpuscles only respond to mechanical stimuli, not to any other type of stimulus — this is a good example of how receptors only respond to specific stimuli.

Tip: Stretch-mediated sodium ion channels get their name because they only open and let sodium ions pass through when they're stretched.

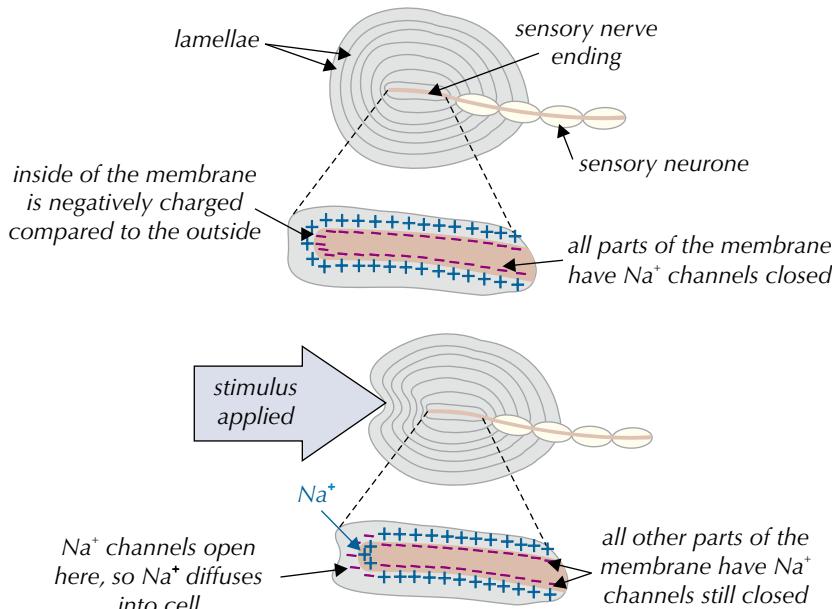


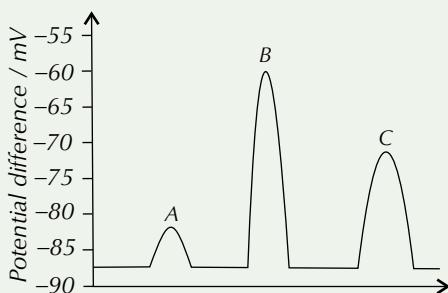
Figure 4: A Pacinian corpuscle at rest (top) and during stimulation (bottom).

Practice Questions — Application

Q1 For a particular receptor cell, an action potential is triggered when the generator potential reaches -60 mV .

- What name is given to the value at which an action potential will be triggered?

The graph below shows generator potentials in the receptor cell.



- Which curve shows a generator potential that would trigger an action potential? Give a reason for your answer.
- What is the resting potential of this receptor cell?

Q2 Suggest how a person's perception of touch might be affected by drugs that block stretch-mediated sodium ion channels in cell membranes.

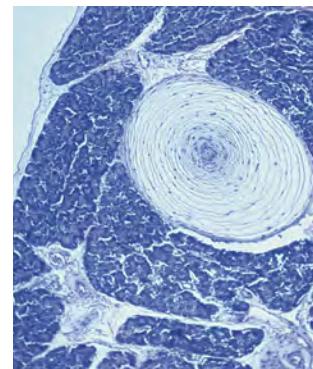


Figure 5: Light micrograph showing a section of a Pacinian corpuscle at rest.

Tip: The bigger the stimulus (i.e. the more pressure that's applied), the more sodium channels open. This creates a bigger generator potential, which is more likely to reach the threshold and cause an action potential.

Photoreceptors

Photoreceptors are receptors in your eye that detect light. Light enters the eye through the pupil, and the amount of light that enters is controlled by the muscles of the iris. Light rays are focused by the lens onto the retina, which lines the inside of the eye. The retina contains the photoreceptor cells. The fovea is an area of the retina where there are lots of photoreceptors. Nerve impulses from the photoreceptor cells are carried from the retina to the brain by the optic nerve, which is a bundle of neurones. Where the optic nerve leaves the eye is called the blind spot — there aren't any photoreceptor cells, so it's not sensitive to light.

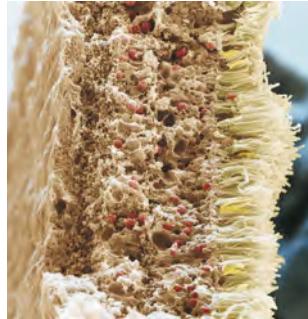


Figure 7: A section through a human retina. Light entering the eye from the left hits the photoreceptors (yellow), which connect to neurones (red).

Tip: Light passes straight through the optic nerve and bipolar neurone to get to the photoreceptors.

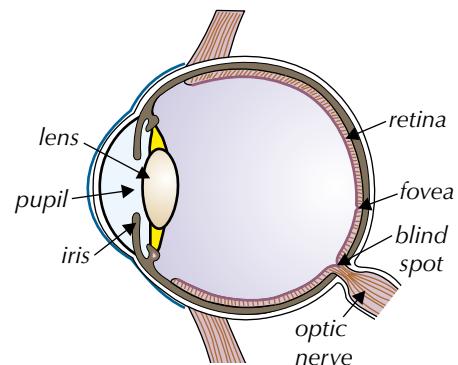


Figure 6: Cross-section of an eye.

How photoreceptors work

Light enters the eye, hits the photoreceptors and is absorbed by light-sensitive optical pigments. Light bleaches the pigments, causing a chemical change and altering the membrane permeability to sodium ions. A generator potential is created and if it reaches the threshold, a nerve impulse is sent along a bipolar neurone. Bipolar neurones connect photoreceptors to the optic nerve, which takes impulses to the brain (see Figure 8).

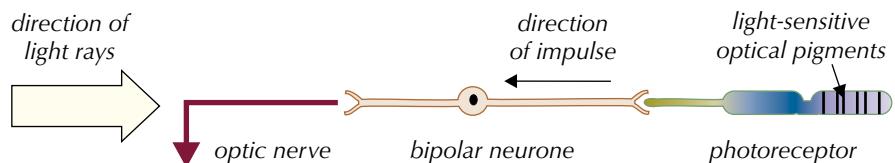


Figure 8: Nervous communication in the eye.

Rods and cones

The human eye has two types of photoreceptor — rods and cones. Rods are mainly found in the peripheral parts of the retina, and cones are mainly found packed together in the fovea — see Figure 9. Rods and cones contain different optical pigments making them sensitive to different wavelengths of light. Rods only give information in black and white (monochromatic vision), but cones give information in colour (trichromatic vision). There are three types of cones each containing a different optical pigment — red-sensitive, green-sensitive and blue-sensitive. When they're stimulated in different proportions you see different colours.

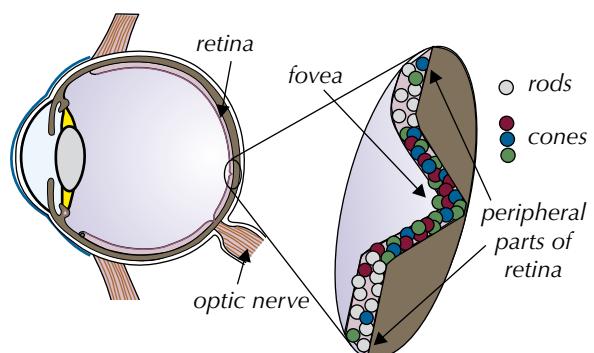
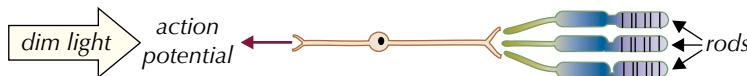


Figure 9: Diagram showing the location of rods and cones.

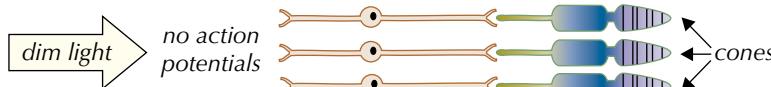
Sensitivity

Rods cells are very sensitive to light (they work well in dim light).

This is because many rods join one bipolar neurone, so many weak generator potentials combine to reach the threshold and trigger an action potential.



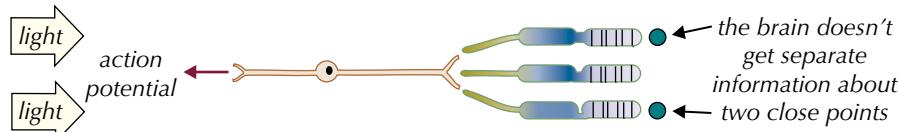
Cones are less sensitive than rods (they work best in bright light). This is because one cone joins one bipolar neurone, so it takes more light to reach the threshold and trigger an action potential.



Visual acuity

Visual acuity is the ability to tell apart points that are close together.

Rods give low visual acuity because many rods join the same bipolar neurone, which means light from two points close together can't be told apart.



Cones give high visual acuity because cones are close together and one cone joins one bipolar neurone. When light from two points hits two cones, two action potentials (one from each cone) go to the brain — so you can distinguish two points that are close together as two separate points.

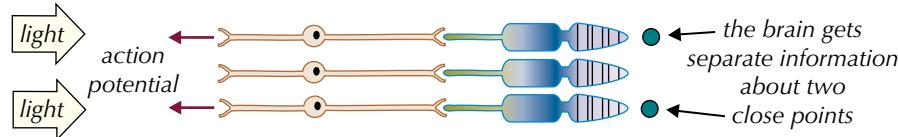


Figure 10: A scanning electron microscope (SEM) image of rod (white) and cone (green) cells in the retina.

Tip: Someone with a low visual acuity will have blurry vision.

Tip: Remember, cones are packed closely together.

Summary table of rods and cones

| Rods | Cones |
|--|------------------------------------|
| Mainly located in the peripheral parts of the retina | Mainly located in the fovea |
| Give information in black and white | Give information in colour |
| Many rods join one bipolar neurone | One cone joins one bipolar neurone |
| High sensitivity to light | Low sensitivity to light |
| Give low visual acuity | Give high visual acuity |

Practice Questions — Fact Recall

- Q1 Explain how a generator potential is produced.
- Q2 What type of stimulus does a Pacinian corpuscle respond to?
- Q3 Describe the structure of a Pacinian corpuscle.
- Q4 Explain how the presence of a stimulus triggers an action potential in a Pacinian corpuscle.
- Q5 Explain why cones give a higher visual acuity than rods.
- Q6 Other than visual acuity, give three differences between rods and cones.

Learning Objectives:

- Know about myogenic stimulation of the heart and the transmission of a subsequent wave of electrical activity.
- Know the roles of the sinoatrial node (SAN), atrioventricular node (AVN) and Purkyne tissue in the bundle of His.
- Know the roles and locations of chemoreceptors and pressure receptors and the roles of the autonomic nervous system and effectors in controlling heart rate.

Specification Reference 3.6.1.3

Tip: You don't need to learn the structure of the nervous system, but understanding it'll help you with the rest of this section.

Tip: To help you remember the difference between the sympathetic and parasympathetic nervous systems, remember: sympathetic for stress, parasympathetic for peacefulness.

Exam Tip

Remember that there's a delay before the AVN reacts. Don't write in the exam that there is a delay in the wave of electrical activity reaching the AVN.

5. Control of Heart Rate

You can't consciously control your heart rate — it's controlled by a part of the nervous system called the autonomic nervous system, which does it for you.

Structure of the nervous system

The nervous system is split into two different systems — the central nervous system (CNS) and the peripheral nervous system. The CNS is made up of the brain and spinal cord, whereas the peripheral nervous system is made up of the neurones that connect the CNS to the rest of the body.

The peripheral nervous system also has two different systems — the somatic and autonomic nervous systems. The somatic nervous system controls conscious activities, e.g. running and playing video games. The autonomic nervous system controls unconscious activities, e.g. digestion.

The autonomic nervous system is split into the sympathetic and parasympathetic nervous systems, which have opposite effects on the body. The sympathetic nervous system is the 'fight or flight' system that gets the body ready for action. The parasympathetic system is the 'rest and digest' system that calms the body down. The autonomic nervous system is involved in the control of heart rate (see next page). The structure of the nervous system is summarised below:

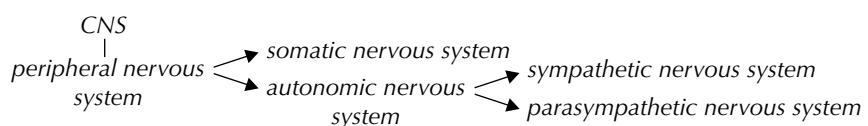


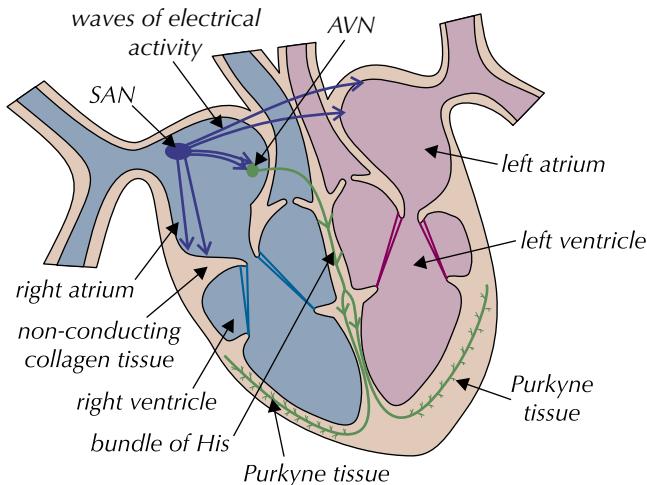
Figure 1: The structure of the nervous system.

Control of heart beat

Cardiac (heart) muscle is '**myogenic**' — this means that it can contract and relax without receiving signals from nerves. This pattern of contractions controls the regular heartbeat.

The process starts in the **sinoatrial node (SAN)**, which is a small mass of tissue in the wall of the right atrium (see Figure 2). The SAN is like a pacemaker — it sets the rhythm of the heartbeat by sending out regular waves of electrical activity to the atrial walls. This causes the right and left atria to contract at the same time. A band of non-conducting collagen tissue prevents the waves of electrical activity from being passed directly from the atria to the ventricles. Instead, these waves of electrical activity are transferred from the SAN to the **atrioventricular node (AVN)**.

The AVN is responsible for passing the waves of electrical activity on to the bundle of His. But, there's a slight delay before the AVN reacts, to make sure the atria have emptied before the ventricles contract. The **bundle of His** is a group of muscle fibres responsible for conducting the waves of electrical activity between the ventricles to the apex (bottom) of the heart. The bundle splits into finer muscle fibres in the right and left ventricle walls, called the **Purkyne tissue**. The Purkyne tissue carries the waves of electrical activity into the muscular walls of the right and left ventricles, causing them to contract simultaneously, from the bottom up.



Tip: Remember the route of the waves of electrical activity by Silly Ants Have Pants — SAN, AVN, bundle of His, Purkyne fibres.

Figure 2: The pathway of electrical activity in the heart.

Communication between the heart and brain

The SAN generates electrical impulses that cause the cardiac muscles to contract. The rate at which the SAN fires (i.e. heart rate) is unconsciously controlled by a part of the brain called the medulla.

Animals need to alter their heart rate to respond to internal stimuli, e.g. to prevent fainting due to low blood pressure or to make sure the heart rate is high enough to supply the body with enough oxygen. Internal stimuli are detected by pressure receptors and chemical receptors:

- There are **pressure receptors** called baroreceptors in the aorta and carotid arteries. They're stimulated by high and low blood pressure.
- There are chemical receptors called **chemoreceptors** in the aorta, the carotid arteries and in the medulla. They monitor the oxygen level in the blood and also carbon dioxide and pH (which are indicators of O₂ level).

Electrical impulses from receptors are sent to the medulla along sensory neurones. The medulla processes the information and sends impulses to the SAN along sympathetic or parasympathetic neurones.

Control of heart rate in response to different stimuli

1. High blood pressure

Baroreceptors detect high blood pressure and send impulses along sensory neurones to the medulla, which sends impulses along parasympathetic neurones. These secrete acetylcholine, which binds to receptors on the SAN. This causes the heart rate to slow down in order to reduce blood pressure back to normal.

2. Low blood pressure

Baroreceptors detect low blood pressure and send impulses along sensory neurones to the medulla, which sends impulses along sympathetic neurones. These secrete noradrenaline, which binds to receptors on the SAN. This causes the heart rate to speed up in order to increase blood pressure back to normal.

Tip: The medulla's full name is the medulla oblongata.

Tip: The carotid arteries are major arteries in the neck.

Tip: Acetylcholine is a type of neurotransmitter. (see page 335 for more).

Tip: Noradrenaline is another type of neurotransmitter.

Tip: The effectors in all of these situations are the cardiac muscles of the heart.

Tip: Low blood O₂, high CO₂ or low blood pH levels are a result of increased respiration.

3. High blood O₂, low CO₂ or high blood pH levels

Chemoreceptors detect chemical changes in the blood and send impulses along sensory neurones to the medulla, which sends impulses along parasympathetic neurones. These secrete acetylcholine, which binds to receptors on the SAN. This causes the heart rate to decrease in order to return oxygen, carbon dioxide and pH levels back to normal.

4. Low blood O₂, high CO₂ or low blood pH levels

Chemoreceptors detect chemical changes in the blood and send impulses along sensory neurones to the medulla, which sends impulses along sympathetic neurones. These secrete noradrenaline, which binds to receptors on the SAN. This causes the heart rate to increase in order to return oxygen, carbon dioxide and pH levels back to normal.

The control of heart rate by the medulla is summarised in Figure 3.

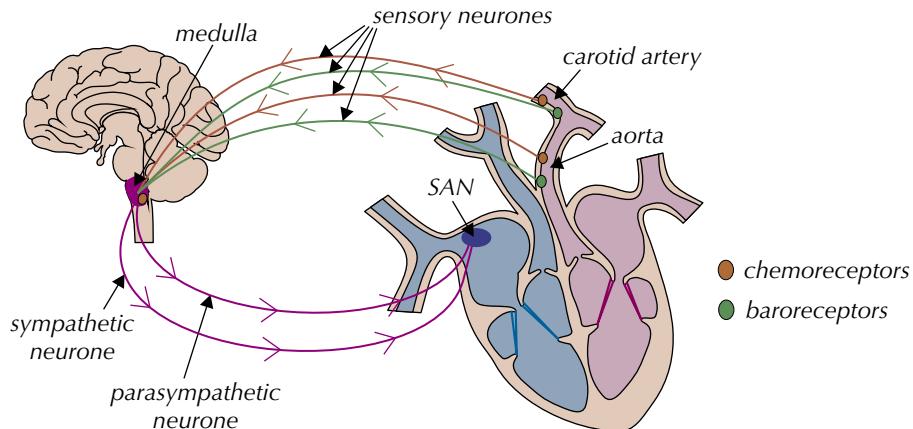


Figure 3: The control of heart rate.

Practice Question — Application

Q1 Anaemia is a condition in which the oxygen carrying capacity of the blood is reduced.

Use your knowledge of the control of heart rate to explain why a person with anaemia is likely to have a more rapid heart rate than someone without anaemia.

Tip: There are lots of receptors in the body that detect changes in blood chemistry — these questions refer to the ones that are used in controlling heart rate.

Practice Questions — Fact Recall

Q1 a) What is the overall role of the autonomic nervous system?
b) Name the two divisions of the autonomic nervous system.

Q2 Heart muscle is described as being 'myogenic'.
What does this mean?

Q3 What is the role of the sinoatrial node (SAN)?

Q4 Name the part of the brain that controls heart rate.

Q5 a) What type of receptor detects a fall in blood pressure?
b) Where are these receptors located in the body?

Section Summary

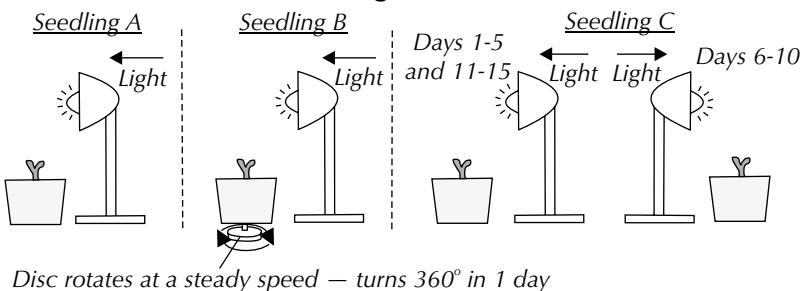
Make sure you know...

- That organisms increase their chance of survival by responding to changes in their environment.
- That simple responses keep mobile organisms in a favourable environment and can be tactic (directional movement in response to a stimulus) or kinetic (non-directional movement in response to a stimulus).
- How to investigate animal responses using a choice chamber or maze, e.g. the movement of woodlice in a choice chamber under different environmental conditions (Required Practical 10).
- That receptors detect stimuli.
- That the coordinator (e.g. the CNS) processes information from receptors, formulates an appropriate response to the stimulus and passes it to an effector (e.g. a muscle), which then produces a response.
- That simple reflexes are rapid, involuntary responses to stimuli which help protect the body from damage. They involve three neurones — a sensory neurone (that connects the receptor to the CNS), a relay neurone (in the spinal cord) and a motor neurone (that connects the CNS to the effector).
- That a tropism is the response of a flowering plant to a change in the environment (a positive tropism is a growth towards a stimulus and a negative tropism is a growth away from a stimulus). These changes are a result of specific hormone-like growth factors that move from growing regions to other tissues and speed up or slow down growth.
- That high concentrations of indoleacetic acid (IAA) promote cell elongation in shoots and inhibit cell elongation in roots. IAA controls the direction of plant growth in response to light (phototropism) by increasing in concentration in shaded parts of shoots and roots, and controls the direction of plant growth in response to gravity (gravitropism) by moving to the underside of shoots and roots.
- That a receptor only responds to a specific stimulus and that when a receptor is stimulated it causes a change in the potential difference across a membrane, called a generator potential.
- That a Pacinian corpuscle is a mechanoreceptor that is made up of a sensory nerve ending wrapped in layers of connective tissue called lamellae. Pressure on the lamellae causes stretch-mediated sodium ion channels to deform, allowing sodium ions to diffuse into the cell and establish a generator potential.
- That photoreceptors are found on the retina of the human eye. There are two types which contain different optical pigments — rods (which give information in black and white) and cones (which give information in colour). Rods and cones have different distributions on the retina.
- That many rods join one bipolar neurone, which makes them very sensitive to light but gives a low visual acuity. One cone joins one bipolar neurone, which makes them less sensitive to light than rods but gives a high visual acuity.
- That cardiac (heart) muscle is myogenic, which means it can contract and relax without receiving signals from nerves.
- That the sinoatrial node (SAN) sends out regular waves of electrical activity that spread across the atria causing them to contract. This electrical activity is transferred to the ventricles by the atrioventricular node (AVN), which connects to the bundle of His and the Purkyne tissue. The Purkyne tissue carries the waves of electrical activity to the left and right ventricles, causing them to contract simultaneously.
- That the autonomic nervous system is involved in the control of heart rate. Stimuli detected by pressure and chemical receptors result in the rate of cardiac muscle contraction, and therefore heart rate, being altered.
- That pressure receptors (baroreceptors) are located in the aorta and carotid arteries and cause heart rate to speed up when low blood pressure is detected and slow down when high blood pressure is detected. Chemical receptors (chemoreceptors) are located in the aorta, carotid arteries and medulla and cause heart rate to speed up when low oxygen, high carbon dioxide or low pH levels are detected and slow down when high oxygen, low carbon dioxide or high pH levels are detected.

Exam-style Questions

- 1 Scientists took three Goosegrass seedlings and planted them in individual pots with soil taken from the same source. They let each seedling grow for 15 days in the conditions shown in **Figure 1**.

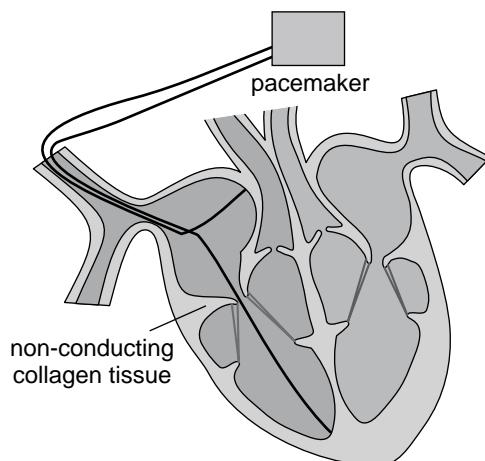
Figure 1



- 1.1 Suggest what response the scientists were testing with this experiment. (1 mark)
- 1.2 The scientists didn't include a negative control in their experiment. Describe the conditions that should have been used for a seedling acting as a negative control. (2 marks)
- 1.3 Suggest why the scientists used soil taken from the same source for each seedling. (1 mark)
- 1.4 Describe and explain the pattern of growth in the three plants you would expect to see by the end of the experiment. (3 marks)
- 1.5 Explain the role of growth factors in controlling the direction of growth in this experiment. (3 marks)
- 2 Protanopia is a type of colour blindness where the photoreceptor that perceives red light is absent.
- 2.1 Explain how photoreceptors in the eye enable coloured light to be perceived. (4 marks)
- 2.2 Explain why having protanopia doesn't necessarily affect the ability to see in low light conditions. (5 marks)
- 2.3 Pupils respond to light intensity by a reflex action which causes the diameter of the pupil to change. The pupil widens in low intensity light and contracts in bright light, as a result of muscles in the eye relaxing and contracting. Suggest how this reflex protects the body from damage. (2 marks)
- 2.4 Describe the pathway of nervous communication in the pupil reflex when bright light is shone in the eyes. (3 marks)

- 3** Some people suffer from a condition called third-degree atrioventricular (AV) block — the waves of electrical activity from the atrioventricular node (AVN) are not relayed to the ventricles. A pacemaker can be fitted to take over this role. **Figure 2** shows a heart with a pacemaker attached.

Figure 2



- 3.1** The pacemaker is programmed to have a delay between receiving waves of electrical activity from the SAN and producing an electrical impulse in the ventricles. Explain the purpose of this delay. **(1 mark)**
- 3.2** Explain the role of the non-conducting collagen tissue. **(1 mark)**
- 3.3** Explain how waves of electrical activity from the AVN lead to blood being pumped out of the heart in a healthy person. **(3 marks)**
- 3.4** AV block can lead to an abnormally slow heart rate. Patients suspected of having the condition are asked about any medication they are taking when being assessed by a doctor.
Drugs called beta-blockers block the action of noradrenaline.
Suggest why a doctor might ask if these drugs are being taken. **(3 marks)**
- 3.5** Third-degree AV block is a complete block of the function of the AVN. Second-degree AV block is a partial blockage which causes a delay in the time taken for the wave of electrical activity to pass through the AVN. People with second-degree AV block may experience dizziness or fainting. Suggest why this might be the case. **(3 marks)**
- 3.6** Where are the chemoreceptors that detect low oxygen levels located? **(1 mark)**

Learning Objectives:

- Understand how a resting potential is established in terms of differential membrane permeability, electrochemical gradients and the movement of sodium and potassium ions.
- Understand how changes in membrane permeability lead to depolarisation and the generation of an action potential.
- Understand the nature and importance of the refractory period in producing discrete impulses and in limiting the frequency of impulse transmission.
- Understand the all-or-nothing principle of an action potential.
- Know the structure of a myelinated motor neurone.
- Understand how an action potential is passed along non-myelinated and myelinated axons, resulting in nerve impulses.
- Know how myelination, saltatory conduction, axon diameter and temperature affect the speed of conductance.

Specification Reference 3.6.2.1

Tip: Remember, sodium-potassium pumps are SOPI — Sodium Out, Potassium In.

1. Neurones

Nervous impulses are the electrical charges transmitted along a neurone. They're created by the movement of sodium and potassium ions.

The resting membrane potential

In a neurone's resting state (when it's not being stimulated), the outside of the membrane is positively charged compared to the inside. This is because there are more positive ions outside the cell than inside. So the membrane is polarised — there's a difference in charge (called a potential difference or voltage) across it. The voltage across the membrane when it's at rest is called the resting potential — it's about -70 mV (millivolts).

Movement of sodium and potassium ions

The resting potential is created and maintained by **sodium-potassium pumps** and **potassium ion channels** in a neurone's membrane (see Figure 1).

- Sodium-potassium pumps use **active transport** to move three sodium ions (Na^+) out of the neurone for every two potassium ions (K^+) moved in. ATP is needed to do this.
- Potassium ion channels allow **facilitated diffusion** of potassium ions (K^+) out of the neurone, down their concentration gradient.

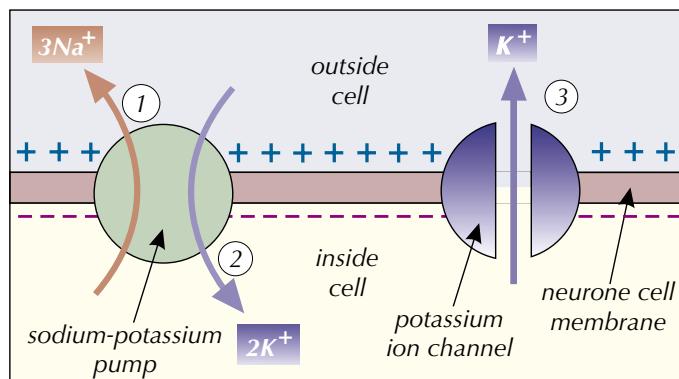


Figure 1: Movement of sodium and potassium ions across a resting cell membrane.

1. The sodium-potassium pumps move sodium ions out of the neurone, but the membrane isn't permeable to sodium ions, so they can't diffuse back in. This creates a sodium ion **electrochemical gradient** (a concentration gradient of ions) because there are more positive sodium ions outside the cell than inside.
2. The sodium-potassium pumps also move potassium ions in to the neurone.
3. When the cell's at rest, most potassium ion channels are open. This means that the membrane is permeable to potassium ions, so some diffuse back out through potassium ion channels.

Even though positive ions are moving in and out of the cell, in total more positive ions move out of the cell than enter. This makes the outside of the cell positively charged compared to the inside.

Action potentials

When a neurone is stimulated, other ion channels in the cell membrane, called sodium ion channels, open. If the stimulus is big enough, it'll trigger a rapid change in potential difference. This causes the cell membrane to become **depolarised** (it's no longer polarised). The sequence of events that happens is known as an action potential — see Figure 2.

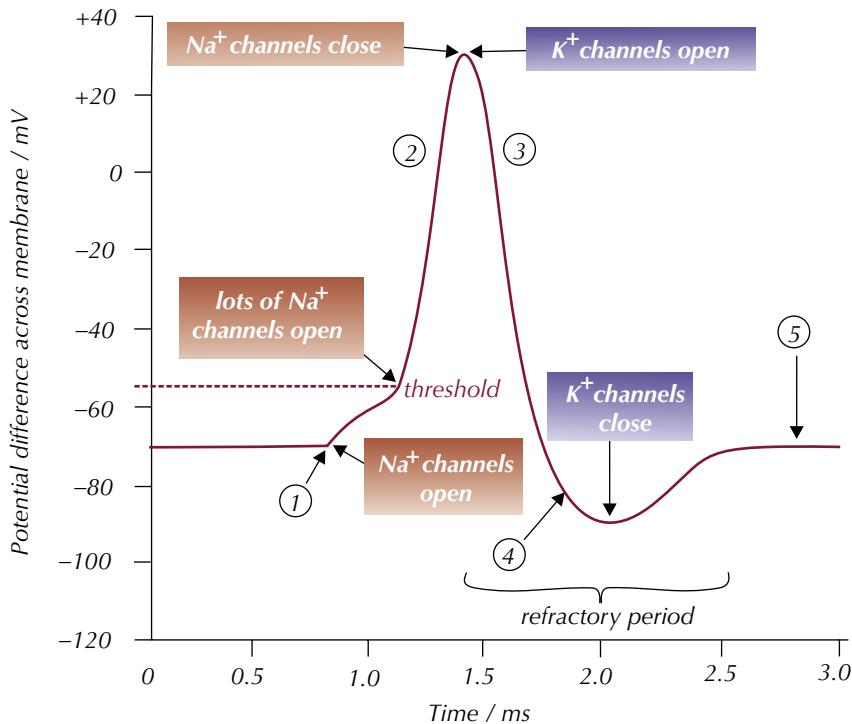


Figure 2: A graph to show the changes in potential difference across a neurone cell membrane during an action potential.

1. **Stimulus** — this excites the neurone cell membrane, causing sodium ion channels to open. The membrane becomes more permeable to sodium, so sodium ions diffuse into the neurone down the sodium ion electrochemical gradient. This makes the inside of the neurone less negative.
2. **Depolarisation** — if the potential difference reaches the threshold (around -55 mV), more sodium ion channels open. More sodium ions diffuse into the neurone.
3. **Repoliarisation** — at a potential difference of around $+30 \text{ mV}$ the sodium ion channels close and potassium ion channels open. The membrane is more permeable to potassium so potassium ions diffuse out of the neurone down the potassium ion concentration gradient. This starts to get the membrane back to its resting potential.
4. **Hyperpolarisation** — potassium ion channels are slow to close so there's a slight 'overshoot' where too many potassium ions diffuse out of the neurone. The potential difference becomes more negative than the resting potential (i.e. less than -70 mV).
5. **Resting potential** — the ion channels are reset. The sodium-potassium pump returns the membrane to its resting potential by pumping sodium ions out and potassium ions in, and maintains the resting potential until the membrane's excited by another stimulus.

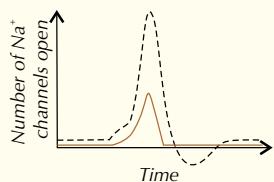
Tip: These sodium ion channels are voltage-gated — they only open when the potential difference reaches a certain voltage.

Tip: The sodium-potassium pump, potassium ion channel and sodium ion channel are all types of transport protein.

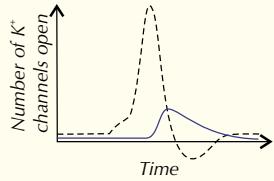
Exam Tip
You don't have to learn these mV values for your exams — they're only approximate and vary from neurone to neurone.

Tip: ms = milliseconds, 1000 ms = 1 second.

Tip: The graph below shows when the sodium ion channels (orange) are open during an action potential (dotted line):



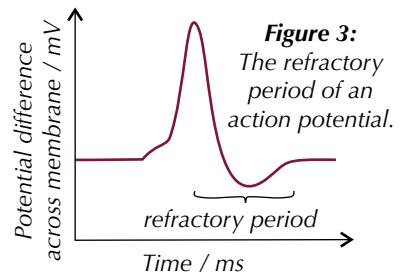
And this graph shows when the potassium ion channels (blue) are open:



Tip: During repolarisation the sodium channels have to close or the membrane will remain depolarised.

The refractory period

After an action potential, the neurone cell membrane can't be excited again straight away. This is because the ion channels are recovering and they can't be made to open — sodium ion channels are closed during repolarisation and potassium ion channels are closed during hyperpolarisation. This period of recovery is called the refractory period (see Figure 3).



The refractory period acts as a time delay between one action potential and the next. This makes sure that action potentials don't overlap but pass along as discrete (separate) impulses. The refractory period also means that there's a limit to the frequency at which the nerve impulses can be transmitted, and that action potentials are unidirectional (they only travel in one direction).

Tip: A wave of depolarisation is like a Mexican wave travelling through a crowd — sodium ions rushing inwards causes a wave of activity along the membrane.

Tip: The electrical impulse can be said to 'propagate' along the neurone. This just describes the wave-like movement of the action potential.

Waves of depolarisation

When an action potential happens, some of the sodium ions that enter the neurone diffuse sideways. This causes sodium ion channels in the next region of the neurone to open and sodium ions diffuse into that part. This causes a wave of depolarisation to travel along the neurone. The wave moves away from the parts of the membrane in the refractory period because these parts can't fire an action potential.

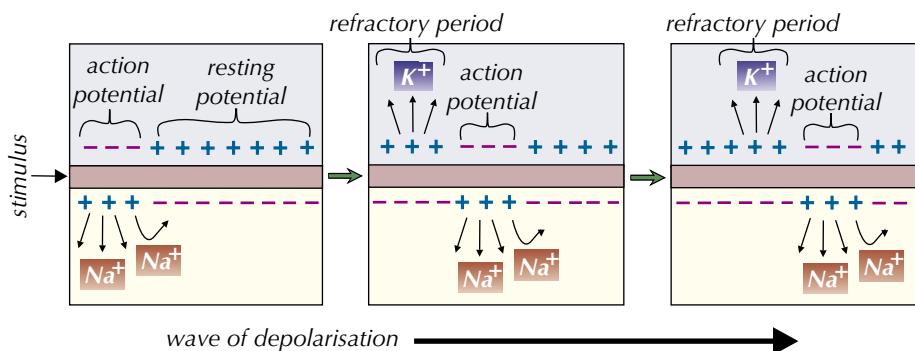


Figure 4: The movement of ions across a neurone cell membrane during a wave of depolarisation.

All-or-nothing principle

Tip: The all-or-nothing principle stops the brain from getting over-stimulated by not responding to very small stimuli.

Once the threshold is reached, an action potential will always fire with the same change in voltage, no matter how big the stimulus is. If the threshold isn't reached, an action potential won't fire (see Figure 5). This is the **all-or-nothing** nature of action potentials.

A bigger stimulus won't cause a bigger action potential but it will cause them to fire more frequently (see Figure 6).

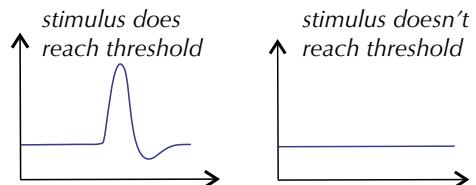


Figure 5: An action potential only fires if the stimulus reaches the threshold.

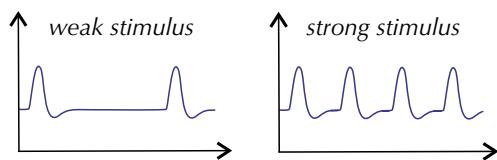


Figure 6: A bigger stimulus causes more frequent action potentials.

Speed of conduction

Three factors affect the speed of conduction of action potentials:

1. Myelination

Some neurones, including many motor neurones, are myelinated — they have a **myelin sheath** (see Figure 8). The myelin sheath is an electrical insulator. In the peripheral nervous system (see page 324), the sheath is made of a type of cell called a **Schwann cell**. Between the Schwann cells are tiny patches of bare membrane called the **nodes of Ranvier**. Sodium ion channels are concentrated at the nodes of Ranvier.

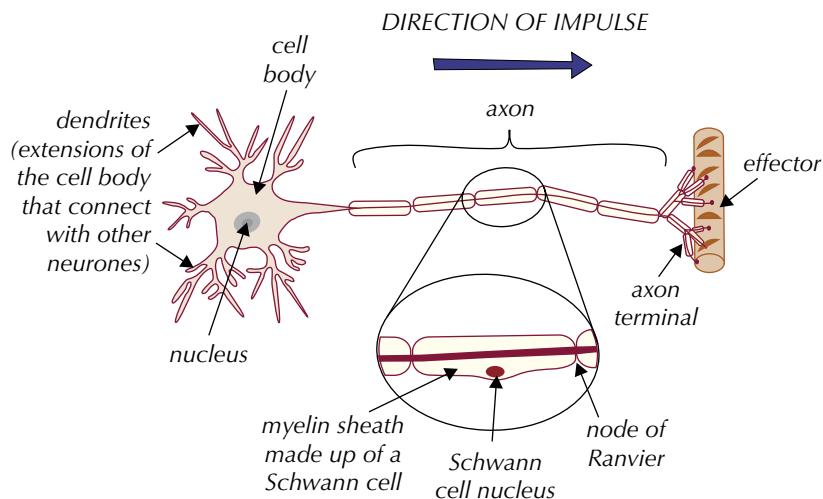


Figure 8: Structure of a myelinated motor neurone.



Figure 7: A cross-section through a myelinated neurone. The myelin sheath (orange/brown) surrounds the axon (dark brown).

Exam Tip

You need to learn the structure of a myelinated motor neurone for your exams.

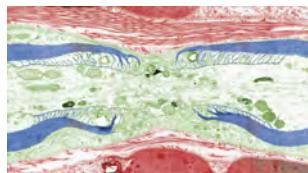


Figure 9: A section through a myelinated neurone. The myelin sheath appears blue — the area where there is no myelin sheath is a node of Ranvier.

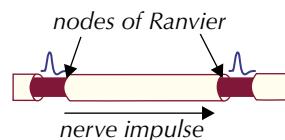


Figure 10: Saltatory conduction along a myelinated neurone.

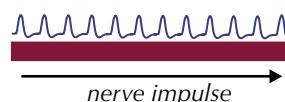


Figure 11: Conduction along a non-myelinated neurone.

Tip: If you imagine a Mexican wave travelling through a crowd, then saltatory conduction is like every tenth person doing the wave instead of everyone doing the wave — so it travels much faster.

Tip: The pumps and channels that move ions across the membrane are proteins, so these will denature at high temperatures.

2. Axon diameter

In a non-myelinated neurone, the impulse travels as a wave along the whole length of the axon membrane — so you get depolarisation along the whole length of the membrane (see Figure 11). This is slower than saltatory conduction (although it's still pretty quick).

In a non-myelinated neurone, the impulse travels as a wave along the whole length of the axon membrane — so you get depolarisation along the whole length of the membrane (see Figure 11). This is slower than saltatory conduction (although it's still pretty quick).

3. Temperature

The speed of conduction increases as the temperature increases too, because ions diffuse faster. The speed only increases up to around 40 °C though — after that the proteins begin to denature and the speed decreases.

Practice Questions — Application

Tip: Remember, the potential difference is the voltage across the membrane.

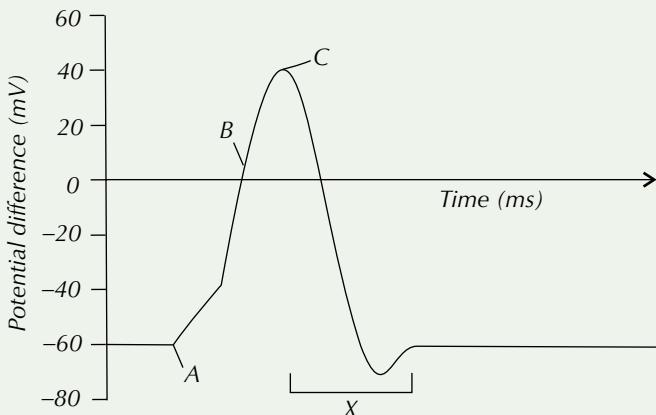
Exam Tip

You might be asked questions like this in your exams, i.e. ones that get you to explain the shape of the graph on page 331. Make sure you really understand what's happening at each point.

Exam Tip

Always be clear in your exam answers as to whether you're talking about sodium ions (Na^+) or potassium ions (K^+) — don't just write 'sodium', 'potassium' or 'ions'.

The graph below shows the changes in potential difference across a neurone cell membrane during an action potential.



- Q1 Describe the different events occurring at points A, B and C.
- Q2 What is the threshold level for this action potential?
- Q3 What is the resting potential of this neurone cell membrane?
- Q4 a) Explain the shape of the curve during the period marked X.
b) What name is given to the period marked X?
- Q5 How would the graph look if a bigger stimulus triggered the action potential? Explain your answer.

Practice Questions — Fact Recall

Tip: The sodium ion channels are closed when the cell's at rest — they only open following the arrival of a stimulus.

Exam Tip

In your exams, be careful not to use phrases like 'ions move across the membrane' — you need to make it clear whether they're moving into or out of the cell.

- Q1 Which two proteins in a neurone's cell membrane are responsible for creating and maintaining the resting membrane potential?
- Q2 Following a stimulus, explain how the opening of sodium ion channels affects the potential difference across a neurone cell membrane.
- Q3 Describe and explain the movement of sodium ions if the potential difference across a neurone cell membrane reaches the threshold level.
- Q4 a) After an action potential, why can't the neurone cell membrane be excited again straight away?
b) What three effects does this have on the conduction of action potentials along a neurone?
- Q5 Explain how waves of depolarisation are produced.
- Q6 Describe the structure of a myelinated neurone in the peripheral nervous system.
- Q7 How does conduction along a myelinated neurone differ compared to conduction along a non-myelinated neurone?
- Q8 Give two factors, other than myelination, that affect the conduction of action potentials.

2. Synaptic Transmission

If you've ever wanted to know more about neurones and how they pass on information, well now's your chance...

Synapses and neurotransmitters

A synapse is the junction between a neurone and another neurone, or between a neurone and an effector cell, e.g. a muscle or gland cell. The tiny gap between the cells at a synapse is called the synaptic cleft. The presynaptic neurone (the one before the synapse) has a swelling called a synaptic knob. This contains synaptic vesicles filled with chemicals called neurotransmitters — see Figure 1.

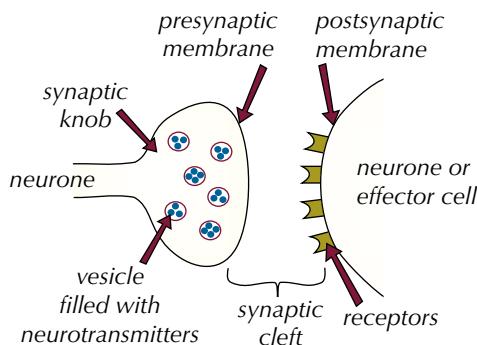


Figure 1: The structure of a typical synapse.

Effect of an action potential

When an action potential reaches the end of a neurone it causes neurotransmitters to be released into the synaptic cleft. They diffuse across to the postsynaptic membrane (the one after the synapse) and bind to specific receptors. When neurotransmitters bind to receptors they might trigger an action potential (in a neurone), cause muscle contraction (in a muscle cell), or cause a hormone to be secreted (from a gland cell).

Because the receptors are only on the postsynaptic membranes, synapses make sure impulses are **unidirectional** — the impulse can only travel in one direction. Neurotransmitters are removed from the cleft so the response doesn't keep happening, e.g. they're taken back into the presynaptic neurone or they're broken down by enzymes (and the products are taken into the neurone).

Acetylcholine

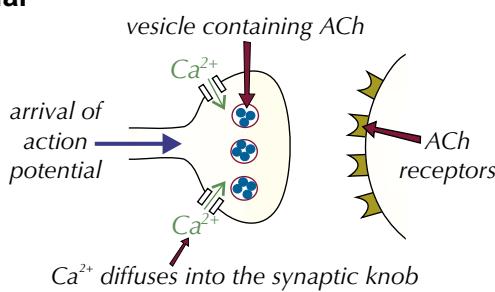
There are many different neurotransmitters. You need to know about one called **acetylcholine (ACh)**, which binds to cholinergic receptors. Synapses that use acetylcholine are called **cholinergic synapses**.

Cholinergic synapses

This is how a nerve impulse is transmitted across a cholinergic synapse:

1. Arrival of an action potential

An action potential arrives at the synaptic knob of the presynaptic neurone. The action potential stimulates voltage-gated calcium ion channels in the presynaptic neurone to open. Calcium ions (Ca^{2+}) diffuse into the synaptic knob. (They're pumped out afterwards by active transport.)



Learning Objectives:

- Know the detailed structure of a synapse.
- Understand the sequence of events involved in transmission across a cholinergic synapse in sufficient detail to be able to explain unidirectionality, inhibition by inhibitory synapses, and spatial and temporal summation.
- Know the detailed structure of a neuromuscular junction.
- Be able to compare transmission across a cholinergic synapse and across a neuromuscular junction.
- Be able to predict and explain the effects of specific drugs on a synapse, when provided with information.

Specification Reference 3.6.2.2



Figure 2: A synaptic knob (yellow) containing vesicles (large red circles).

Tip: Noradrenaline is another example of a neurotransmitter — see pages 325–326.

Tip: Voltage-gated ion channels are channels that only open when the potential difference across a membrane reaches a certain voltage.

Exam Tip

"The influx of calcium ions..." means that the calcium ions have flowed into the synaptic knob. You'll lose out on marks in the exam if you talk about an influx of calcium ions out of the synaptic knob.

Tip: Exocytosis is the process by which a vesicle inside a cell moves to the cell-surface membrane, fuses with the membrane and releases its contents outside the cell.

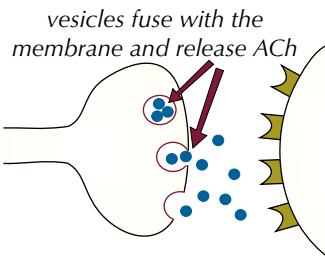
Tip: Look back at page 331 if you need a reminder of how action potentials are generated.

Tip: 'Depolarise' means making the potential difference across the neurone membrane more positive. 'Hyperpolarise' means making the potential difference across the membrane more negative. (See page 331 for more.)

Tip: Acetylcholine is both an excitatory and an inhibitory neurotransmitter

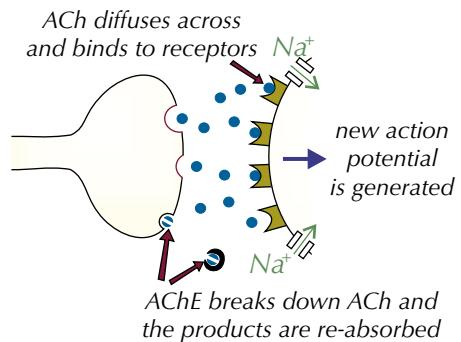
2. Fusion of the vesicles

The influx of calcium ions into the synaptic knob causes the synaptic vesicles to fuse with the presynaptic membrane. The vesicles release the neurotransmitter acetylcholine (ACh) into the synaptic cleft by exocytosis.



3. Diffusion of ACh

ACh diffuses across the synaptic cleft and binds to specific cholinergic receptors on the postsynaptic membrane. This causes sodium ion channels in the postsynaptic membrane to open. The influx of sodium ions into the postsynaptic membrane causes depolarisation. An action potential on the postsynaptic membrane is generated if the threshold is reached. ACh is removed from the synaptic cleft so the response doesn't keep happening. It's broken down by an enzyme called acetylcholinesterase (AChE) and the products are re-absorbed by the presynaptic neurone and used to make more ACh.



Excitatory and inhibitory neurotransmitters

Neurotransmitters can be excitatory, inhibitory or both. Excitatory neurotransmitters depolarise the postsynaptic membrane, making it fire an action potential if the threshold is reached.

Example

Acetylcholine is an excitatory neurotransmitter (it binds to cholinergic receptors to cause an action potential in the postsynaptic membrane) at cholinergic synapses in the CNS and at neuromuscular junctions (see page 338).

Inhibitory neurotransmitters hyperpolarise the postsynaptic membrane (make the potential difference more negative), preventing it from firing an action potential.

Examples

- GABA is an inhibitory neurotransmitter — when it binds to its receptors it causes potassium ion channels to open on the postsynaptic membrane, hyperpolarising the neurone.
- Acetylcholine is an inhibitory neurotransmitter at cholinergic synapses in the heart. When it binds to receptors here, it can cause potassium ion channels to open on the postsynaptic membrane, hyperpolarising it.

A synapse where inhibitory neurotransmitters are released from the presynaptic membrane following an action potential is called an **inhibitory synapse**.

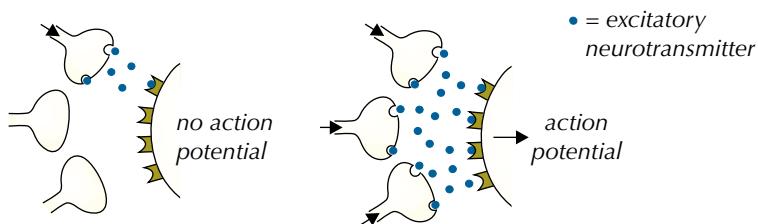
Summation at synapses

If a stimulus is weak, only a small amount of neurotransmitter will be released from a neurone into the synaptic cleft. This might not be enough to excite the postsynaptic membrane to the threshold level and stimulate an action potential. Summation is where the effect of neurotransmitters released from many neurones (or one neurone that's stimulated a lot in a short period of time) is added together. It means synapses accurately process information, finely tuning the response. There are two types of summation:

Tip: Summation is where the sum total of lots of smaller impulses triggers an action potential.

1. Spatial summation

Spatial summation is where two or more presynaptic neurones release their neurotransmitters at the same time onto the same postsynaptic neurone. The small amount of neurotransmitter released from each of these neurones can be enough altogether to reach the threshold in the postsynaptic neurone and trigger an action potential — see Figure 3.



Tip: Remember, only excitatory neurotransmitters can trigger an action potential (see previous page).

Figure 3: One presynaptic neurone only releases a few neurotransmitters (left) but three presynaptic neurones release enough to trigger an action potential (right).

If some neurones release an inhibitory neurotransmitter then the total effect of all the neurotransmitters might be no action potential — see Figure 4.



Figure 4: If some presynaptic neurones release inhibitory neurotransmitters, it might prevent an action potential from being triggered (right).

2. Temporal summation

Temporal summation is where two or more nerve impulses arrive in quick succession from the same presynaptic neurone. This makes an action potential more likely because more neurotransmitter is released into the synaptic cleft — see Figure 5.

Tip: Impulses have to follow each other very quickly, otherwise the neurotransmitter will be removed from the cleft before it's reached a level high enough to trigger an action potential.

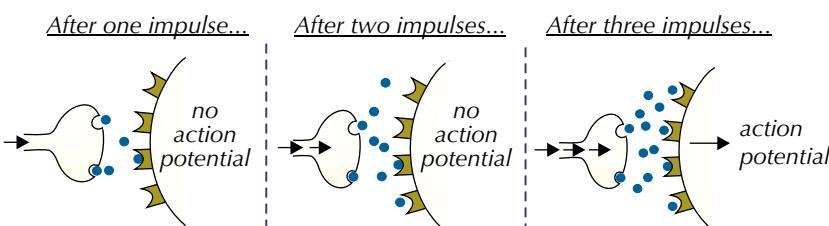


Figure 5: The effects of temporal summation at a synapse.

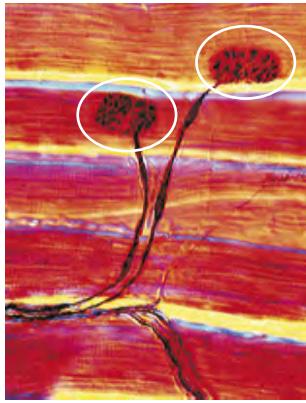


Figure 6: A light micrograph showing neuromuscular junctions (circled) in skeletal muscle.

Exam Tip

You need to be able to compare transmission across a neuromuscular junction and a cholinergic synapse (see pages 335-336 for a reminder of cholinergic synapses).

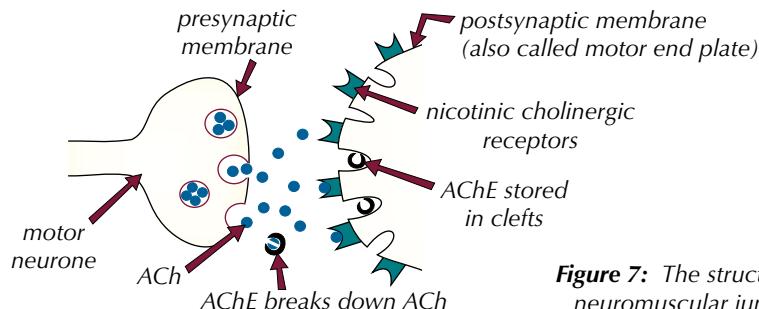


Figure 7: The structure of a neuromuscular junction.

Neuromuscular junctions work in basically the same way as cholinergic synapses, i.e. they both release ACh from vesicles in the presynaptic membrane, ACh then diffuses across the synaptic cleft and binds to cholinergic receptors on the postsynaptic membrane, and this triggers an action potential if the threshold is reached. In both types of synapse, ACh is broken down in the synaptic cleft by the enzyme acetylcholinesterase (AChE).

There are a few differences between the two types of synapse too.

For example, at a neuromuscular junction:

- The postsynaptic membrane has lots of folds that form clefts. These clefts store AChE.
- The postsynaptic membrane has more receptors than other synapses.
- ACh is always excitatory, so when a motor neurone fires an action potential, it normally triggers a response in a muscle cell. This isn't always the case for a synapse between two neurones.

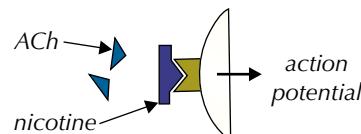
Drugs at synapses

Exam Tip

You don't have to learn the names of all the drugs given here (and on the next page), but make sure you understand how they affect synaptic transmission. In the exam, you could be given information about a particular drug you've not come across before and be asked to predict the effects the drug would have at a synapse.

Example

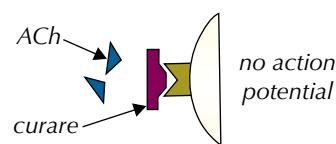
Nicotine mimics acetylcholine so binds to nicotinic cholinergic receptors in the brain.



Some drugs block receptors so they can't be activated by neurotransmitters (these drugs are called antagonists). This means fewer receptors (if any) can be activated.

Example

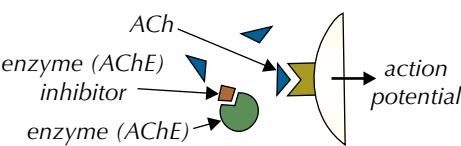
Curare blocks the effects of acetylcholine by blocking nicotinic cholinergic receptors at neuromuscular junctions, so muscle cells can't be stimulated. This results in the muscle being paralysed.



Some drugs inhibit the enzyme that breaks down neurotransmitters (they stop it from working). This means there are more neurotransmitters in the synaptic cleft to bind to receptors and they're there for longer.

Example

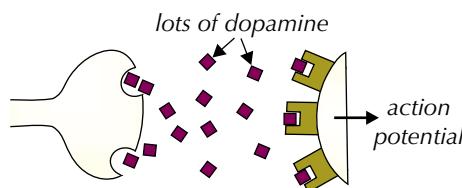
Nerve gases stop acetylcholine from being broken down in the synaptic cleft. This can lead to loss of muscle control.



Some drugs stimulate the release of neurotransmitter from the presynaptic neurone so more receptors are activated.

Example

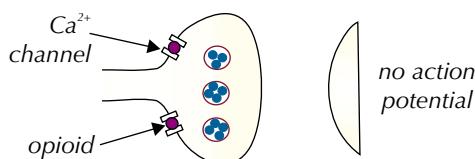
Amphetamines force a neurotransmitter called dopamine out of synaptic vesicles and into the synaptic cleft. This increases the effect of dopamine, e.g. it increases alertness.



Some drugs inhibit the release of neurotransmitters from the presynaptic neurone so fewer receptors are activated.

Example

Opioids block calcium ion channels in the presynaptic neurone. This means fewer vesicles fuse with the presynaptic membrane so less neurotransmitter is released.



Practice Questions — Application

- Q1 Lambert–Eaton myasthenic syndrome (LEMS) is an autoimmune disorder where antibodies are formed against calcium ion channels in the neuromuscular junction, preventing the channels from working properly. Suggest what the main symptom of LEMS might be. Explain your answer.
- Q2 Endorphins are endogenous opioid peptides that function as inhibitory neurotransmitters. Endorphins bind to opioid receptors on neurones that transmit pain signals.
- Suggest what effect endorphins have on the sensation of pain. Explain your answer.
 - Morphine is an opioid drug that's very similar in structure to an endorphin molecule. Suggest what effect taking morphine will have on a person's sensation of pain. Explain your answer.
- Q3 Acetylcholine (ACh) is involved in many functions in the body, including saliva production. Carbachol is a drug that binds and activates cholinergic receptors. Predict the effect of carbachol on saliva production and explain your answer.

Tip: An autoimmune disease is where a person's immune system mistakes their own cells for pathogens, so it starts to attack them.

Tip: Endogenous just means it's produced naturally by the body.

Practice Questions — Fact Recall

Q1 The diagram on the right shows a cholinergic synapse. Name the structures labelled A to G on the diagram.

Q2 Give three types of cell that have receptors for neurotransmitters.

Q3 Explain why impulses at a synapse are unidirectional.

Q4 At a cholinergic synapse:

a) Describe and explain the movement of calcium ions following the arrival of an action potential at a presynaptic neurone.

b) Explain how acetylcholine (ACh) leaves the presynaptic neurone and causes an action potential in the postsynaptic neurone.

Q5 Why is it important that ACh is removed from the synaptic cleft by being broken down by the enzyme AChE?

Q6 What effect does an inhibitory neurotransmitter have on a postsynaptic membrane?

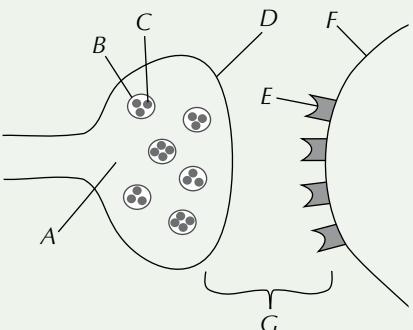
Q7 Explain how an action potential may be more likely as a result of:

a) spatial summation,

b) temporal summation.

Q8 What is a neuromuscular junction?

Q9 Give three ways in which a neuromuscular junction is similar to a cholinergic synapse.



Tip: Don't get the presynaptic and postsynaptic neurones mixed up — remember 'pre' means before and 'post' means after.

3. Muscle Structure

Muscles are effectors — they contract in response to nervous impulses. To understand how muscles contract, first you need to know about their structure...

Types of muscle

There are three different types of muscle in the body:

- **Smooth muscle** contracts without conscious control. It's found in walls of internal organs (apart from the heart), e.g. stomach, intestine and blood vessels.
- **Cardiac muscle** contracts without conscious control (like smooth muscle) but it's only found in the heart.
- **Skeletal muscle** (also called striated, striped or voluntary muscle) is the type of muscle you use to move, e.g. the biceps and triceps move the lower arm.

You need to know the ins and outs of skeletal muscle for the exams...

Learning Objectives:

- Understand that muscles act in antagonistic pairs against an incompressible skeleton.
- Know the gross and microscopic structure of skeletal muscle.
- Know the ultrastructure of a myofibril.

Specification Reference 3.6.3

Role of skeletal muscle

Skeletal muscles are attached to bones by tendons. Ligaments attach bones to other bones, to hold them together. Pairs of skeletal muscles contract and relax to move bones at a joint — the bones of the skeleton are incompressible (rigid) so they act as levers, giving the muscles something to pull against.

Tip: Ligaments are bands of strong connective tissue.

Antagonistic pairs

Muscles that work together to move a bone are called antagonistic pairs. The contracting muscle is called the agonist and the relaxing muscle is called the antagonist.

Example — Biceps and triceps

The bones of your lower arm are attached to a biceps muscle and a triceps muscle by tendons. The biceps and triceps work together to move your arm — as one contracts, the other relaxes.

When your biceps contracts your triceps relaxes. This pulls the bone so your arm bends (flexes) at the elbow — see Figure 1. Here, the biceps is the agonist and the triceps is the antagonist.

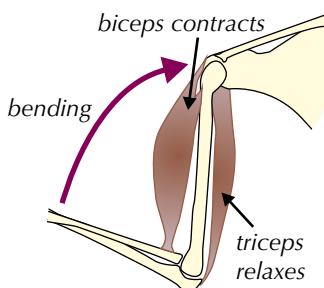


Figure 1: Arm bending.

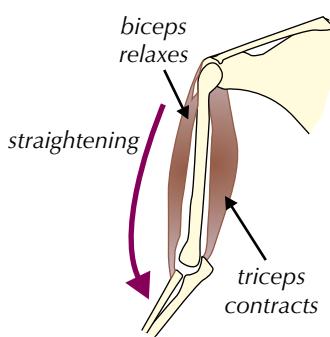


Figure 2: Arm straightening.

When your triceps contracts your biceps relaxes. This pulls the bone so your arm straightens (extends) at the elbow — see Figure 2. Here, the triceps is the agonist and the biceps is the antagonist.

Tip: Muscles work in pairs because they can only pull when they contract — they can't push.

Structure of skeletal muscle

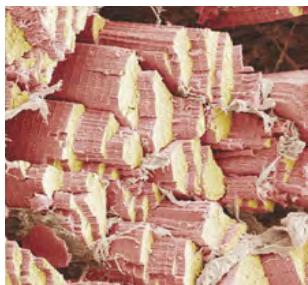


Figure 3: A scanning electron microscope (SEM) image of a section of muscle fibre with myofibrils (pink and yellow) bundled together.

Tip: Myofibrils are organelles within the cell (muscle fibre).

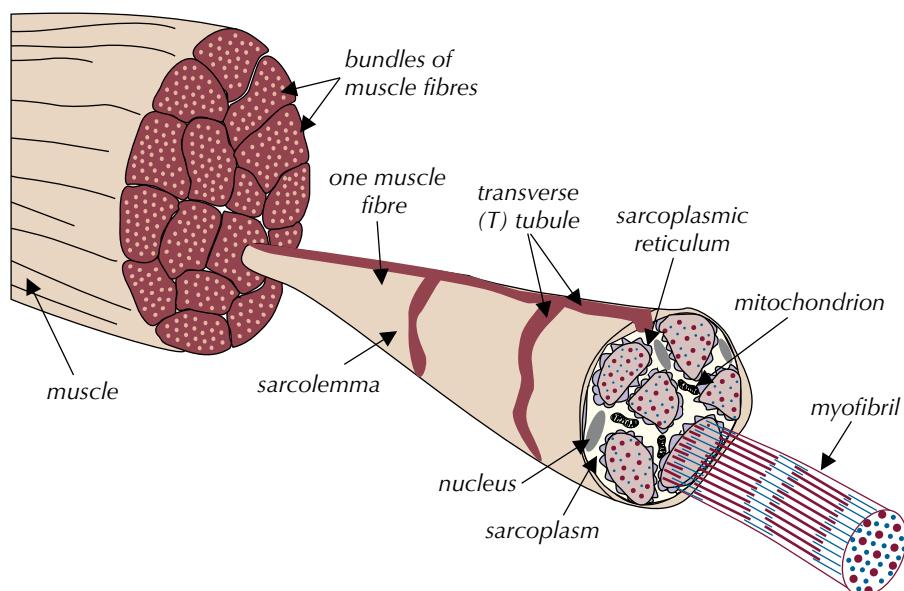


Figure 4: Diagram showing the structure of skeletal muscle and a muscle fibre.

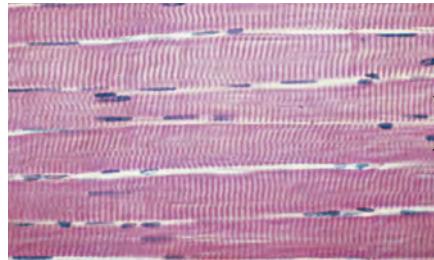
Tip: Longitudinal cross-sections are taken along the length of a structure, whereas transverse cross-sections cut through the structure at a right angle to its length.

Examination under an optical microscope

You could look at skeletal muscle under an optical microscope. What you see will depend on how the sample has been stained and whether you're looking at a longitudinal or transverse cross-section.

Example

This photomicrograph shows a longitudinal cross-section of skeletal muscle. You can see six muscle fibres. The blue parts are nuclei — there are many in each muscle fibre. The cross-striations (alternating darker and lighter pink stripes) are the A-bands and I-bands of the myofibrils, see next page.



Myofibrils

Myofibrils contain bundles of thick and thin myofilaments that move past each other to make muscles contract. The thick myofilaments are made of the protein **myosin** and the thin myofilaments are made of the protein **actin**.

If you look at a myofibril under an electron microscope, you'll see a pattern of alternating dark and light bands (see Figures 5 and 6). Dark bands contain the thick myosin filaments and some overlapping thin actin filaments — these are called A-bands. Light bands contain thin actin filaments only — these are called I-bands.

Tip: You need to know the 'ultrastructure' of a myofibril — this just means the fine structure that can only be seen using an electron microscope.

A myofibril is made up of many short units called **sarcomeres**. The ends of each sarcomere are marked with a Z-line. In the middle of each sarcomere is an M-line. The M-line is the middle of the myosin filaments. Around the M-line is the H-zone. The H-zone only contains myosin filaments.

Tip: To remember which band is which, think: **dark = A-bands** and **light = I-bands**.

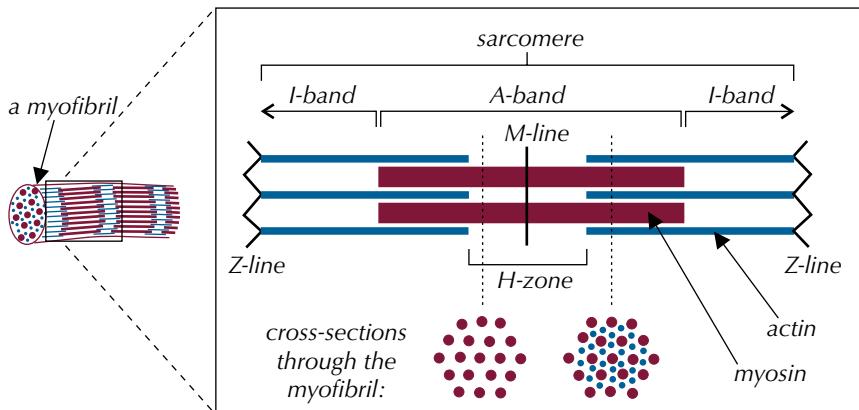


Figure 5: The structure of a sarcomere — a unit of a myofibril.

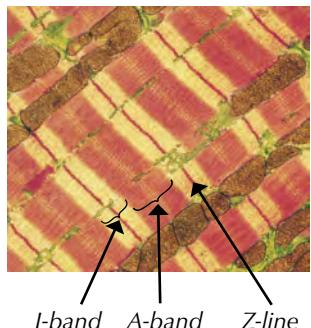


Figure 6: A transmission electron microscope (TEM) of myofibrils showing the banding of myosin (red) and actin (yellow).

The sliding filament theory

Muscle contraction is explained by the sliding filament theory. This is where myosin and actin filaments slide over one another to make the sarcomeres contract — the myofilaments themselves don't contract. The simultaneous contraction of lots of sarcomeres means the myofibrils and muscle fibres contract. Sarcomeres return to their original length as the muscle relaxes.

Tip: There's a lot more detail on muscle contraction coming up (see pages 345-347).

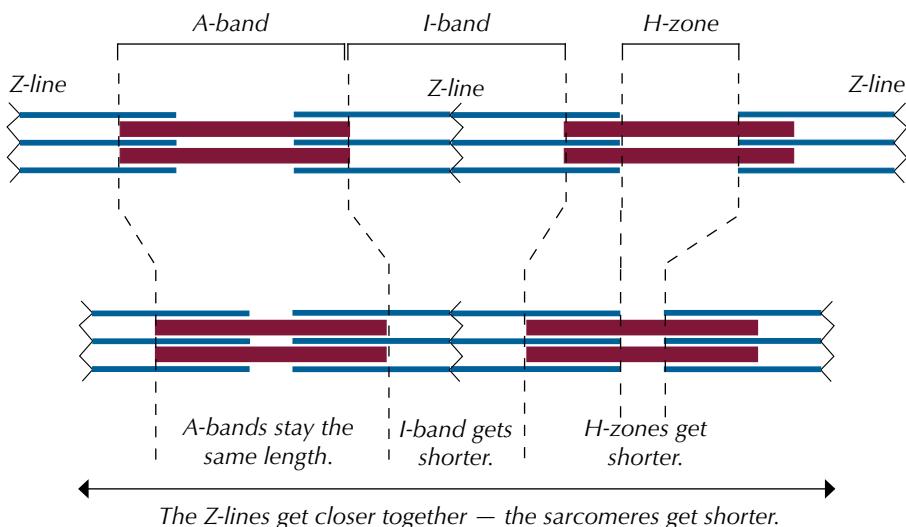


Figure 7: Sarcomeres during relaxation (top) and contraction (bottom).

Tip: A bands are the only ones that stay the same length.

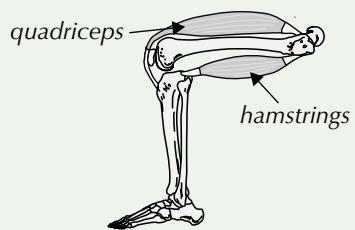
Practice Questions — Application

Tip: If you're struggling with Q1, look closely at which bones the muscles are attached to and think how the contraction of each muscle would move the bones.

Tip: The diagrams in Q2 might look a bit odd at first, but with a bit of logical thinking you should be able to work out the answers.

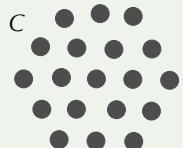
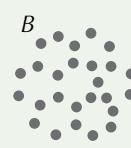
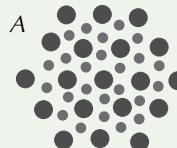
It might help if you sketch out the sarcomere structure with the bands on, so you can see what's happening.

- Q1 The quadriceps and hamstrings are antagonistic muscles that are attached to bones in the leg. In the diagram on the right, which muscle is acting as the antagonist? Explain your answer.



- Q2 Cross sections from three different sites along a sarcomere are shown below. Which cross-section(s) could be from:

- a) an I-band?
- b) an M-line?
- c) an A-band?
- d) a Z-line?



- Q3 The lengths of three different sections of a sarcomere were measured when a rabbit muscle was relaxed. These values are given in the first column of the table below. Work out which other set of values in the table (options 1-3) shows the lengths of the sections when the muscle was contracted. Explain your answer.

| | Relaxed (μm) | Option 1 (μm) | Option 2 (μm) | Option 3 (μm) |
|--------|---------------------|----------------------|----------------------|----------------------|
| A-band | 1.5 | 1.5 | 1.2 | 1.5 |
| I-band | 0.8 | 0.5 | 0.5 | 1 |
| H-zone | 0.7 | 0.2 | 0.7 | 0.2 |

Practice Questions — Fact Recall

- Q1 What is meant by an 'antagonistic pair' of muscles?
- Q2 Describe the structure and function of T-tubules.
- Q3 Why do muscle fibres contain lots of mitochondria?
- Q4 Describe the structure of an A-band in a myofibril and describe its appearance under an electron microscope.
- Q5 What is the sliding filament theory of muscle contraction?

4. Muscle Contraction

So now you know all about the structure of muscle it's time to find out just how it contracts...

Myosin and actin filaments

Muscle contraction involves myosin and actin filaments sliding over one another. Here's a bit more detail about the two types of filament:

Myosin filaments

Myosin filaments have globular heads that are hinged, so they can move back and forth. Each myosin head has a binding site for actin and a binding site for ATP — see Figure 1.

Actin filaments

Actin filaments have binding sites for myosin heads, called actin-myosin binding sites. Another protein called **tropomyosin** is found between actin filaments. It helps myofilaments move past each other (see Figure 1).

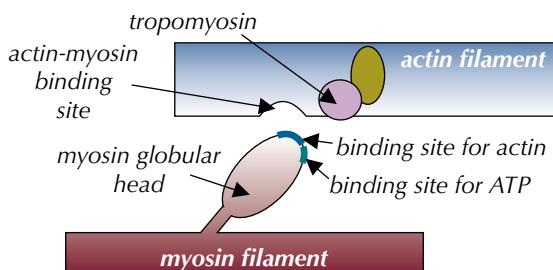


Figure 1: The structure of myosin and actin filaments.

Binding sites in resting muscles

For myosin and actin filaments to slide past each other, the myosin head needs to bind to the actin-myosin binding site on the actin filament. In a resting (unstimulated) muscle the actin-myosin binding site is blocked by tropomyosin — see Figure 2. This means myofilaments can't slide past each other because the myosin heads can't bind to the actin filaments.

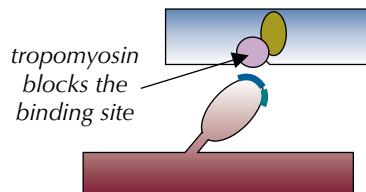


Figure 2: Actin and myosin filaments in resting muscle.

Learning Objectives:

- Understand the roles of calcium ions and tropomyosin in the cycle of actin-myosin cross bridge formation.
- Understand the roles of actin, myosin, calcium ions and ATP in myofibril contraction.
- Understand the role of ATP and phosphocreatine in muscle contraction.
- Know the structure, location and general properties of slow and fast skeletal muscle fibres.

Specification Reference 3.6.3

Tip: This diagram has been simplified — tropomyosin molecules actually form part of a long chain that coils round the actin filament.

The process of muscle contraction

Arrival of an action potential

When an action potential from a motor neurone stimulates a muscle cell, it depolarises the sarcolemma. Depolarisation spreads down the T-tubules to the sarcoplasmic reticulum. This causes the sarcoplasmic reticulum to release stored calcium ions (Ca^{2+}) into the sarcoplasm. This influx of calcium ions into the sarcoplasm triggers muscle contraction.

Calcium ions bind to a protein attached to tropomyosin, causing the protein to change shape. This pulls the attached tropomyosin out of the actin-myosin binding site on the actin filament. This exposes the binding site, which allows the myosin head to bind. The bond formed when a myosin head binds to an actin filament is called an **actin-myosin cross bridge** — see Figure 3 on the next page.

Tip: If you can't remember your sarcolemma from your sarcoplasmic reticulum then take a look back at page 342.

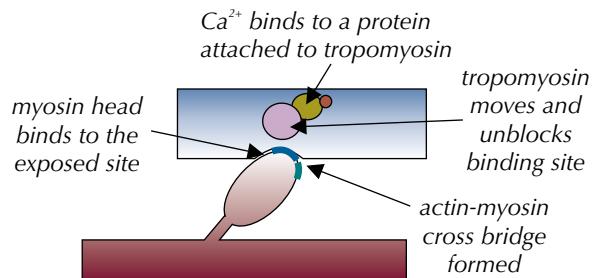


Figure 3: Formation of an actin-myosin cross bridge.

Movement of the actin filament

Calcium ions also activate the enzyme ATP hydrolase, which hydrolyses (breaks down) ATP (into ADP + P_i) to provide the energy needed for muscle contraction. The energy released from ATP causes the myosin head to bend, which pulls the actin filament along in a kind of rowing action (see Figure 4).

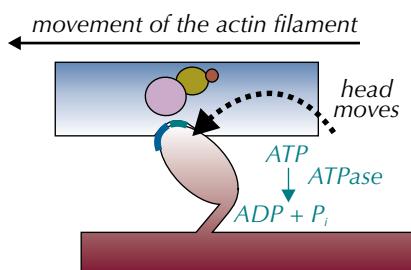


Figure 4: Movement of the myosin head.

Breaking of the cross bridge

Another ATP molecule provides the energy to break the actin-myosin cross bridge, so the myosin head detaches from the actin filament after it's moved. The myosin head then returns to its starting position, and reattaches to a different binding site further along the actin filament — see Figure 5. A new actin-myosin cross bridge is formed and the cycle is repeated (attach, move, detach, reattach to new binding site...).

Many actin-myosin cross bridges form and break very rapidly, pulling the actin filament along — which shortens the sarcomere, causing the muscle to contract. The cycle will continue as long as calcium ions are present.

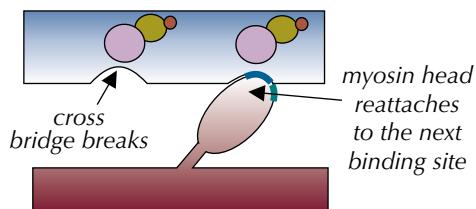


Figure 5: Myosin head forms a new actin-myosin cross bridge.

Return to resting state

When the muscle stops being stimulated, calcium ions leave their binding sites and are moved by active transport back into the sarcoplasmic reticulum (this needs ATP too).

This causes the tropomyosin molecules to move back, so they block the actin-myosin binding sites again — see Figure 6. Muscles aren't contracted because no myosin heads are attached to actin filaments (so there are no actin-myosin cross bridges). The actin filaments slide back to their relaxed position, which lengthens the sarcomere.

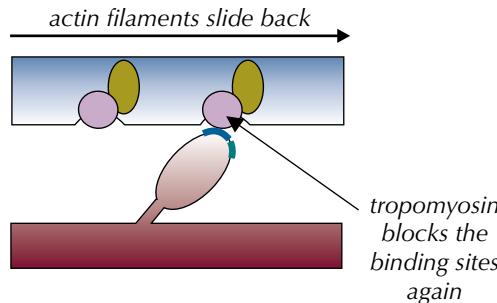
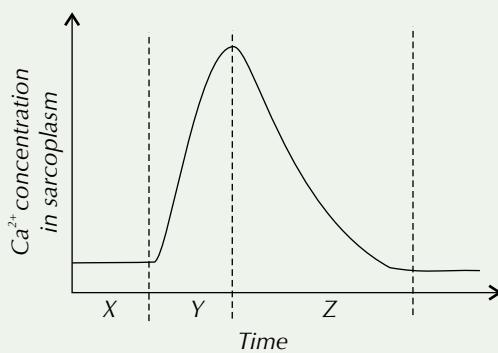


Figure 6: Blocking of the actin-myosin binding sites as the muscle returns to its resting state.

Practice Questions — Application

- Q1 The graph below shows the calcium ion concentration in the sarcoplasm of a muscle fibre over time.



- During what time period (X, Y or Z):
 - is the muscle fibre the longest length? Explain your answer.
 - would Ca^{2+} ions be bound to the protein attached to tropomyosin? Explain your answer.
 - would ATP hydrolase be activated? Explain your answer.
- Describe the movement of calcium ions during time period Z.
- Describe the event that causes an increase in Ca^{2+} ions in the sarcoplasm at the beginning of time period Y.

- Q2 Cardiac muscle in the heart has some similarities to skeletal muscle, for example, it has both actin and myosin filaments. Patients who suffer from heart failure may be given positive inotropic agents — these are substances which increase the level of calcium ions in the cytoplasm of muscle cells.

Use your knowledge of muscle contraction to explain why this treatment may be used.

Exam Tip

Remember, it's dead easy to lose marks in the exam by rushing headlong into answering a question without reading it through properly first. Take your time — make sure you understand any information in a table or a graph before attempting the question.

Tip: Heart failure is where the heart is unable to pump enough blood around the body.

Tip: Have a flick back to pages 280-286 if you need to remind yourself of the processes involved in aerobic and anaerobic respiration.

Tip: Many activities use a combination of these systems.

Energy for muscle contraction

So much energy is needed when muscles contract that ATP gets used up very quickly. ATP has to be continually generated so exercise can continue — this happens in three main ways:

1. Aerobic respiration

Most ATP is generated via oxidative phosphorylation in the cell's mitochondria. Aerobic respiration only works when there's oxygen so it's good for long periods of low-intensity exercise, e.g. a long walk.

2. Anaerobic respiration

ATP is made rapidly by glycolysis. The end product of glycolysis is pyruvate, which is converted to lactate by lactate fermentation. Lactate can quickly build up in the muscles and cause muscle fatigue. Anaerobic respiration is good for short periods of hard exercise, e.g. a 400 m sprint.

3. ATP-phosphocreatine (PCr) system

ATP is made by phosphorylating ADP — adding a phosphate group taken from PCr. The equation for this is shown in Figure 7. PCr is stored inside cells and the ATP-PCr system generates ATP very quickly. PCr runs out after a few seconds so it's used during short bursts of vigorous exercise, e.g. a tennis serve. The ATP-PCr system is anaerobic (it doesn't need oxygen) and it's alactic (it doesn't form any lactate).



Figure 7: Phosphorylation of ADP by PCr.

Some of the creatine (Cr) gets broken down into creatinine, which is removed from the body via the kidneys. Creatinine levels can be higher in people who exercise regularly and those with a high muscle mass. High creatinine levels may also indicate kidney damage.

Slow twitch and fast twitch muscle fibres

Skeletal muscles are made up of two types of muscle fibres — slow twitch and fast twitch. Different muscles have different proportions of slow and fast twitch fibres. The two types have different properties:

Slow twitch muscle fibres

Slow twitch muscle fibres contract slowly and can work for a long time without getting tired. This makes them good for endurance activities, e.g. long-distance running and maintaining posture. High proportions of slow twitch muscle fibres are found in the muscles you use for posture, such as the muscles in the back and in the calves.

Energy is released slowly through aerobic respiration (see above) in slow twitch muscle fibres. They have lots of mitochondria and blood vessels to supply the muscles with oxygen. The mitochondria are mainly found near to the edge of muscle fibres, so that there's a short diffusion pathway for oxygen from the blood vessels to the mitochondria.

Slow twitch muscle fibres are also rich in myoglobin, a red-coloured protein that stores oxygen, so they're reddish in colour.

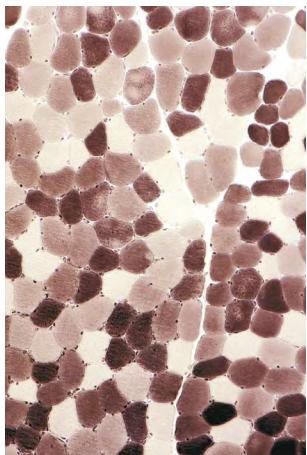


Figure 8: A light micrograph showing a transverse cross-section of a muscle. The dark fibres are slow twitch and the light fibres are fast twitch.

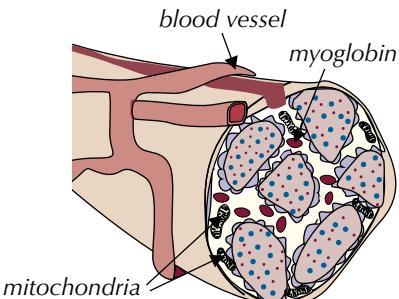


Figure 9: A slow twitch muscle fibre.

Fast twitch muscle fibres

Fast twitch muscle fibres contract very quickly but also get tired quickly. This makes them good for short bursts of speed and power, e.g. sprinting and eye movement. High proportions of fast twitch muscle fibres are found in muscles you use for fast movement, such as the legs, arms and eyes.

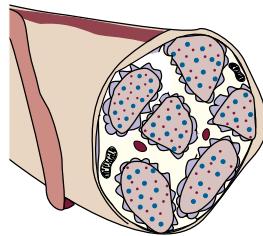


Figure 10:
A fast twitch muscle fibre, with few blood vessels, myoglobin or mitochondria.

Energy is released quickly through anaerobic respiration using glycogen in fast twitch muscle fibres. They also have stores of PCr so that energy can be generated very quickly when needed (see previous page). Fast twitch muscle fibres have few mitochondria or blood vessels. They don't have much myoglobin either, so they can't store much oxygen — this gives them more of a whitish colour.

Tip: Cells are able to store excess glucose as glycogen, which can be converted back into glucose when needed.

Practice Question — Application

- Q1 A marathon runner runs 25 miles at a steady pace. She then speeds up for the 26th mile and sprints the last 385 yards to the finish line.
- Discuss the ways in which the marathon runner is most likely to generate ATP during the course of the race.
 - Is the marathon runner likely to have a greater proportion of fast twitch or slow twitch muscle fibres in her leg muscles? Give a reason for your answer.

Practice Questions — Fact Recall

- Q1 Name a protein found between actin filaments that help myofilaments slide past each other.
- Q2 Explain how calcium ions in the sarcoplasm allow the formation of actin-myosin cross bridges.
- Q3 Describe the role of ATP in muscle contraction.
- Q4
 - Describe how ATP is generated in the ATP-phosphocreatine system.
 - Give one advantage and one disadvantage of generating ATP via the ATP-phosphocreatine system.
 - Give two other ways in which ATP can be generated.
- Q5 Give two ways in which slow twitch muscle fibres are adapted for their function.

Section Summary

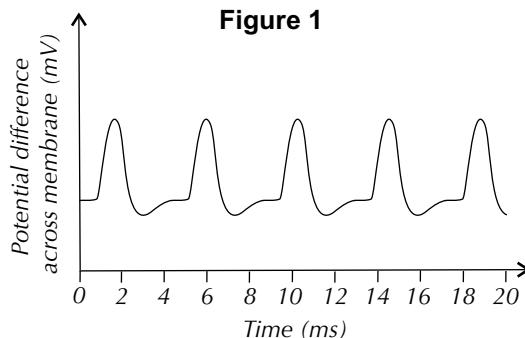
Make sure you know...

- How a resting membrane potential is established and maintained by sodium-potassium pumps and potassium ion channels in a neurone's cell membrane.
- That when sodium ion channels in the membrane open the membrane becomes more permeable to sodium ions and this causes depolarisation (the potential difference of the membrane becomes more positive), resulting in an action potential if the threshold level is reached.
- That the refractory period is when sodium and potassium ion channels can't be made to open again, and that this is important to ensure action potentials form discrete impulses, have a limited frequency and are unidirectional.
- That the all-or-nothing principle means that if the threshold is reached, an action potential will always fire with the same change in voltage and if the threshold isn't reached there'll be no action potential.
- The structure of a myelinated neurone including the dendrites, axon, axon terminal and myelin sheath.
- Why action potentials are passed more quickly along myelinated neurones than unmyelinated neurones.
- That bigger diameters and higher temperatures speed up the rate of conduction along a neurone.
- The detailed structure of a synapse including the synaptic knob, vesicles and postsynaptic membrane.
- That impulses at a synapse are unidirectional because only the postsynaptic membrane has receptors.
- How a nerve impulse is transmitted across a cholinergic synapse, including the role of voltage-gated calcium channels, calcium ions, acetylcholine (ACh), cholinergic receptors on the postsynaptic membrane, sodium ion channels and sodium ions.
- That at inhibitory synapses, inhibitory neurotransmitters are released from the presynaptic neurone, which hyperpolarise the postsynaptic membrane (make the potential difference more negative), preventing it from firing an action potential.
- That spatial summation is the total effect of all the neurotransmitters released from many neurones present at a synapse and that temporal summation is where two or more nerve impulses arrive in quick succession from the same presynaptic neurone.
- The detailed structure of a neuromuscular junction.
- The similarities and differences in transmission across a cholinergic synapse and a neuromuscular junction.
- How to use information given about a drug to predict and explain the effects it will have at a synapse.
- That muscles work in antagonistic pairs to move bones at a joint — as one muscle (the agonist) contracts and pulls the bone, the other muscle (the antagonist) relaxes.
- That skeletal muscle is made up of large bundles of muscle fibres that contain transverse tubules, sarcolemma, sarcoplasm, sarcoplasmic reticulum, myofibrils, and lots of mitochondria and nuclei.
- That myofibrils contain thick myosin and thin actin filaments, and are divided into sarcomeres. A-bands on sarcomeres contain myosin and actin filaments, I-bands only contain actin filaments.
- That, during muscle contraction, calcium ions bind to a protein attached to tropomyosin, which pulls tropomyosin out of the actin-myosin binding site so that actin-myosin cross bridges can be formed.
- How actin, myosin, calcium ions and ATP work together to make a myofibril contract.
- That energy from ATP is used for muscle contraction and that ATP generation may involve the ATP-phosphocreatine (PCr) system.
- That slow twitch muscle fibres are good for endurance activities, release energy slowly through aerobic respiration and are found in muscles such as those used for posture.
- That fast twitch muscle fibres are good for short bursts of speed and power, release energy quickly through anaerobic respiration and are found in muscles, such as those in the eyes and legs, which are used for fast movement.

Exam-style Questions

- 1 Human skeletal muscle is made up of both slow twitch and fast twitch muscle fibres, the proportions of which can vary depending on the location of the muscle and a person's activity levels. Both types of muscle fibres contain GLUT4, a membrane protein that helps glucose to be transported across a plasma membrane.
- 1.1 A person who performs a lot of low-intensity training may have a higher proportion of slow twitch muscle fibres than fast twitch muscle fibres. Suggest a reason for this. (1 mark)
- 1.2 Sprinters often have more fast twitch muscle fibres than marathon runners. Give one function of fast twitch muscle fibres and explain how they are adapted for this function. (3 marks)
- 1.3 During muscle contraction, the content of GLUT4 in muscle cell membranes increases. Explain why. (3 marks)
- 1.4 Muscle contraction involves the movement of actin and myosin filaments. Describe the structure of a myosin filament. (2 marks)

- 2 **Figure 1** shows three action potentials recorded across the membrane of a myelinated axon (axon X).



- 2.1 Explain why the action potentials don't overlap. (3 marks)
- 2.2 If the action potentials continue at the same frequency, calculate the number of action potentials along the axon in 0.5 s. (2 marks)
- 2.3 At the same temperature, another myelinated axon (axon Y) conducted 140 action potentials in 0.5 s. Use your answer to 2.2 to suggest whether axon X or axon Y has the biggest diameter. Explain your answer. (3 marks)

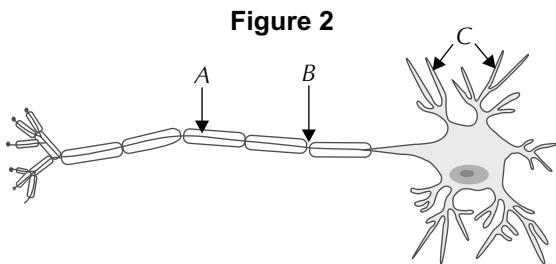
- 3 **Figure 2** shows the structure of a myelinated motor neurone in the peripheral nervous system.

- 3.1 Name the type of cell that forms structure A.

(1 mark)

- 3.2 Name the structures labelled B and C.

(2 marks)



Guillain-Barré syndrome is an autoimmune disease whereby the myelin sheath around certain neurones is damaged.

- 3.3 Explain the function of myelin in a normal motor neurone.

(2 marks)

- 3.4 Use your knowledge of myelination to explain how Guillain-Barré syndrome can result in muscle weakness and paralysis.

(2 marks)

- 4 A bodybuilder lifts weights to increase the size of the muscles in his arms.

- 4.1 Describe how the biceps and triceps muscles work together to bend the arm.

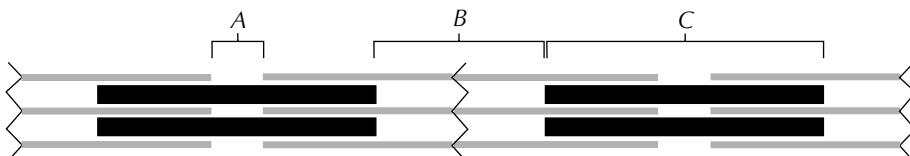
(3 marks)

- 4.2 Explain the role of acetylcholine in causing depolarisation of cells in the biceps muscle following a nervous impulse.

(5 marks)

Figure 3 shows part of a myofibril in the biceps muscle when it is **contracted**.

Figure 3



- 4.3 Name the sections of the myofibril labelled A-C.

(3 marks)

- 4.4 For each of the sections A-C, state how it will appear when the biceps relaxes, compared to how it appears in **Figure 3**.

(2 marks)

The bodybuilder manages to lift an extremely heavy weight with a short burst of explosive power. He can only sustain the lift for a few seconds.

- 4.5 Describe how ATP is likely to be generated in the bodybuilder's arm muscles when he lifts the heavy weight.

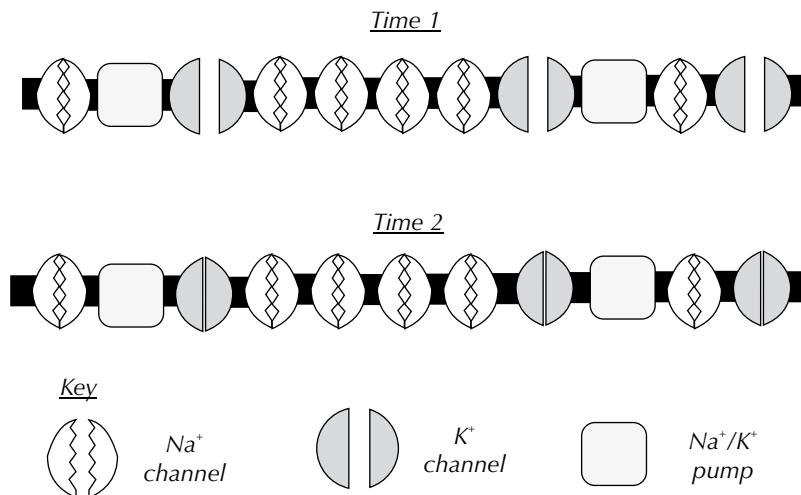
(2 marks)

- 4.6 Give **one** advantage of ATP being generated in this way.

(1 mark)

- 5** **Figure 4** shows a neurone cell membrane at two different times during one action potential.

Figure 4



- 5.1** Describe the stages of the action potential that are occurring at Times 1 and 2. Use evidence from **Figure 4** to support your answer.

(6 marks)

The neurone cell membrane shows sodium-potassium (Na^+/K^+) pumps.

- 5.2** Describe the movement of sodium and potassium ions across a sodium-potassium pump.

(2 marks)

- 5.3** Explain why a sodium-potassium pump is needed by the neurone cell membrane after Time 2.

(2 marks)

- 5.4** Explain how the speed at which the action potential is conducted along the neurone would differ at a temperature of 30°C compared to 20°C .

(2 marks)

An action potential is more likely if two or more nerve impulses arrive in quick succession from the same presynaptic neurone.

- 5.5** What is the name given to this type of summation?

(1 mark)

- 5.6** Explain why this type of summation makes an action potential more likely at a cholinergic synapse.

(3 marks)

- 5.7** Tetrodotoxin is a chemical that blocks sodium ion channels.

Use your knowledge of action potentials to explain the effect that tetrodotoxin is likely to have upon the nervous system.

(3 marks)

Topic 6

C: Homeostasis

Learning Objectives:

- Understand that homeostasis in mammals involves physiological control systems that maintain the internal environment within restricted limits.
- Understand the importance of maintaining a stable core temperature and stable blood pH in relation to enzyme activity.
- Understand the importance of maintaining a stable blood glucose concentration in terms of the water potential of blood and of availability of respiratory substrate.
- Know that negative feedback restores systems to their original level.
- Understand that the possession of separate mechanisms involving negative feedback controls departures in different directions from the original state, giving a greater degree of control.
- Be able to interpret information relating to examples of negative and positive feedback.

Specification Reference 3.6.4.1

1. Homeostasis Basics

The body has some pretty clever systems to control its internal environment...

What is homeostasis?

Changes in your external environment can affect your internal environment — the blood and tissue fluid that surrounds your cells. Homeostasis is the maintenance of a stable internal environment. It involves control systems that keep your internal environment roughly constant (within certain limits). This means your internal environment is kept in a state of dynamic equilibrium (i.e. fluctuating around a normal level). Keeping your internal environment stable is vital for cells to function normally and to stop them being damaged.

The importance of homeostasis

It's particularly important to maintain the right core body temperature and blood pH. This is because temperature and pH affect enzyme activity, and enzymes control the rate of **metabolic reactions** (chemical reactions in living cells). It's also important to maintain the right blood glucose concentration because cells need glucose for energy and blood glucose concentration affects the water potential of blood — see page 356.

Temperature

The rate of metabolic reactions increases when the temperature's increased. More heat means more kinetic energy, so molecules move faster. This makes the substrate molecules more likely to collide with the enzymes' active sites. The energy of these collisions also increases, which means each collision is more likely to result in a reaction.

But, if the temperature gets too high (e.g. over 40 °C), the reaction essentially stops. The rise in temperature makes the enzyme's molecules vibrate more. If the temperature goes above a certain level, this vibration breaks some of the hydrogen bonds that hold the enzyme in its 3D shape. The active site changes shape and the enzyme and substrate no longer fit together. At this point, the enzyme is denatured — it no longer functions as a catalyst (see Figure 1).

If body temperature is too low enzyme activity is reduced, slowing the rate of metabolic reactions. The highest rate of enzyme activity happens at their optimum temperature — about 37 °C in humans (see Figure 1).

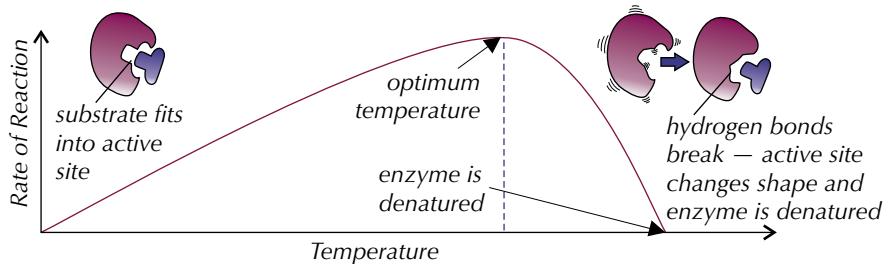


Figure 1: Effect of temperature on the rate of a metabolic reaction.

pH

If blood pH is too high or too low (highly alkaline or acidic) enzymes become denatured (see Figure 2). The ionic bonds and hydrogen bonds that hold them in their 3D shape are broken, so the shape of the enzyme's active site is changed and it no longer works as a catalyst. The highest rate of enzyme activity happens at their optimum pH, so this is when metabolic reactions are fastest. Optimum pH is usually around pH 7 (neutral), but some enzymes work best at other pHs, e.g. enzymes found in the stomach work best at a low pH.

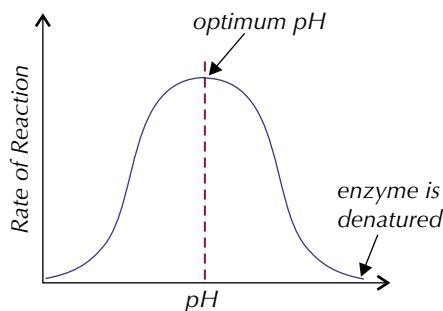


Figure 2: Effect of pH on a metabolic reaction.

Tip: When an enzyme is denatured the reaction may still happen but it'll be too slow for the body's needs.

pH is calculated based on the concentration of hydrogen ions (H^+) in the environment. The greater the concentration of H^+ , the lower the pH will be (and the more acidic the environment). You can work out the pH of a solution using the following equation:

$$\text{pH} = -\log_{10} [\text{H}^+]$$

\log_{10} tells you the pH is expressed on a **logarithmic scale**. You could get asked to use a logarithmic scale in the exam so here's a bit more about them:

A logarithmic scale is a scale that uses the **logarithm** of a number instead of the number itself. Each value on a logarithmic scale using \log_{10} is ten times larger than the value before — so a solution of pH 3 contains ten times more H^+ ions than a solution of pH 4. This is because the concentration of H^+ can vary enormously and so it's easier to compare values on a logarithmic scale. Converting values to a logarithmic scale also makes it easier to plot both very small and very large values (e.g. both 0.1 and 1000) on the same axis of a graph.

$[\text{H}^+]$ is the concentration of hydrogen ions in a solution, measured in mol dm^{-3} . So, if you know the hydrogen ion concentration of a solution, you can calculate its pH by sticking the numbers into the formula above.

Tip: Increasing the number by 1 on a \log_{10} scale is the same as multiplying by 10 on a linear (normal) scale. So the numbers 1, 2, 3 and 4 on a \log_{10} scale represent 10, 100, 1000 and 10,000 on a linear scale:

| | | | | |
|--------------|----|-----|------|--------|
| log scale | 1 | 2 | 3 | 4 |
| linear scale | 10 | 100 | 1000 | 10,000 |

Examples — Maths Skills

1. A solution has a hydrogen ion concentration of 0.01 mol dm^{-3} . Calculate its pH.

$$\begin{aligned}\text{pH} &= -\log_{10} [\text{H}^+] \\ &= -\log_{10} (0.01) \\ &= 2\end{aligned}$$

Just substitute the $[\text{H}^+]$ value into the pH formula and solve.

2. A blood sample has a hydrogen ion concentration of $3.9 \times 10^{-8} \text{ mol dm}^{-3}$. What is the pH of the blood?

$$\begin{aligned}\text{pH} &= -\log_{10} [\text{H}^+] \\ &= -\log_{10} (3.9 \times 10^{-8}) \\ &= 7.4\end{aligned}$$

Tip: Another situation where logarithms come in handy is with microbial growth, where the number of organisms increases exponentially (see page 421).

Tip: The values being expressed on a logarithmic scale are often very large or very small quantities, so standard form is often used. See pages 7 and 8 for more on standard form.

To calculate logarithms you need to use the 'log' button on your calculator. On most calculators, 'log' will stand for \log_{10} , but different calculators work differently so make sure you know how to calculate logs on yours.

Tip: Water potential is the potential (likelihood) of water molecules to diffuse out of or into a solution.

Tip: Glucose is a solute. It lowers the water potential of the blood. If the blood glucose concentration is too high, water molecules move by osmosis from the cells (an area of higher water potential) to the blood (an area of lower water potential).

Tip: Glucose is a respiratory substrate — a substance that can be broken down during respiration to release energy.

Tip: The 'level' in Figure 4 refers to something inside the body that needs to be controlled, e.g. temperature level, pH level, blood glucose level.

Blood glucose concentration

If blood glucose concentration is too high, the water potential of blood is reduced to a point where water molecules diffuse out of cells into the blood by osmosis (the diffusion of water molecules from an area of higher water potential to an area of lower water potential, across a partially permeable membrane). This can cause the cells to shrivel up and die (see Figure 3).

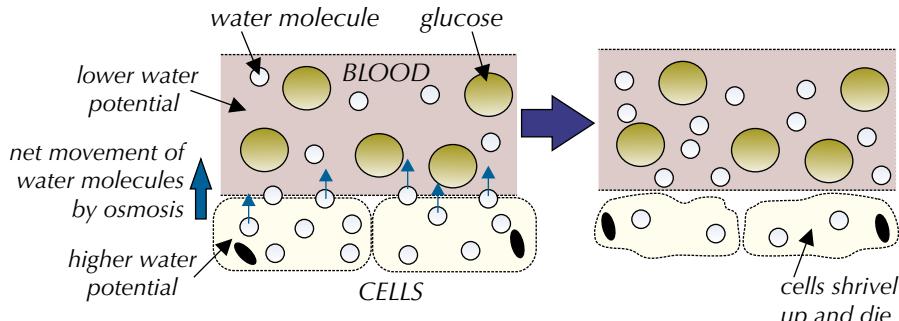


Figure 3: Effect of high blood glucose concentration on cells.

If blood glucose concentration is too low, cells are unable to carry out normal activities because there isn't enough glucose for respiration to provide energy.

Negative feedback

Homeostatic systems involve receptors, a communication system and effectors (see page 314). Receptors detect when a level is too high or too low, and the information's communicated via the nervous system or the hormonal system to effectors. The effectors respond to counteract the change — bringing the level back to normal. The mechanism that restores the level to normal is called a **negative feedback mechanism** — see Figure 4.

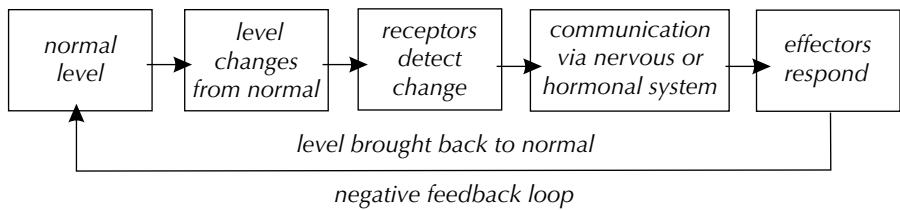


Figure 4: A negative feedback mechanism.

Negative feedback keeps things around the normal level.

Example

Body temperature is usually kept within 0.5 °C above or below 37 °C.

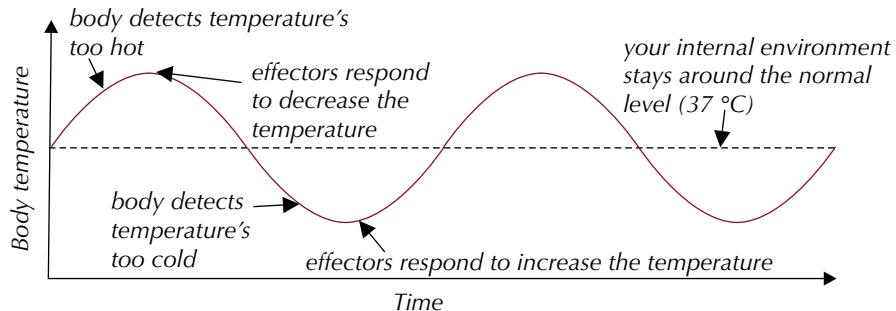


Figure 5: Control of body temperature via negative feedback.

Negative feedback only works within certain limits though — if the change is too big then the effectors may not be able to counteract it, e.g. a huge drop in body temperature caused by prolonged exposure to cold weather may be too large to counteract.

Multiple negative feedback mechanisms

Homeostasis involves multiple negative feedback mechanisms for each thing being controlled. This is because having more than one mechanism gives more control over changes in your internal environment than just having one negative feedback mechanism.

Having multiple negative feedback mechanisms means you can actively increase or decrease a level so it returns to normal, e.g. you have feedback mechanisms to reduce your body temperature and you also have mechanisms to increase it. If you only had one negative feedback mechanism, all you could do would be turn it on or turn it off. You'd only be able to actively change a level in one direction so it returns to normal. Only one negative feedback mechanism means a slower response and less control.

Tip: Think of this as trying to slow down a car with only an accelerator — all you can do is take your foot off the accelerator (you'd have more control with a brake too).

Positive feedback

Some changes trigger a positive feedback mechanism, which amplifies the change. The effectors respond to further increase the level away from the normal level. The mechanism that amplifies a change away from the normal level is called a **positive feedback mechanism** — see Figure 6.

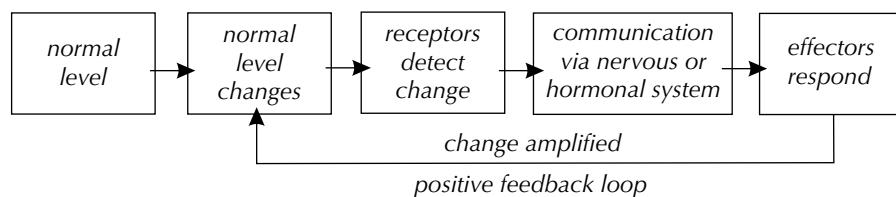


Figure 6: A positive feedback mechanism.

Positive feedback isn't involved in homeostasis because it doesn't keep your internal environment stable. Positive feedback is useful to rapidly activate processes in the body.

Example

During the formation of a blood clot after an injury, platelets become activated and release a chemical — this triggers more platelets to be activated, and so on. This means platelets very quickly form a blood clot at the injury site. (The process ends with negative feedback, when the body detects the blood clot has been formed.)

Breakdown of homeostatic systems

Positive feedback can also happen when a homeostatic system breaks down.

Example

Hypothermia is low body temperature (below 35 °C). It happens when heat's lost from the body quicker than it can be produced. As body temperature falls the brain doesn't work properly and shivering stops — this makes body temperature fall even more (see Figure 8 — next page). Positive feedback takes body temperature further away from the normal level, and it continues to decrease unless action is taken.

Tip: 'Hypo' is often used to describe a condition where something being controlled (in this case body temperature) has fallen below its normal level — 'hyper' is when it's gone above its normal level.



Figure 7: A person with hypothermia could be helped by being wrapped in a foil blanket as shown above. The foil blanket minimises further heat loss from the body so internal temperature should increase back to within normal limits.

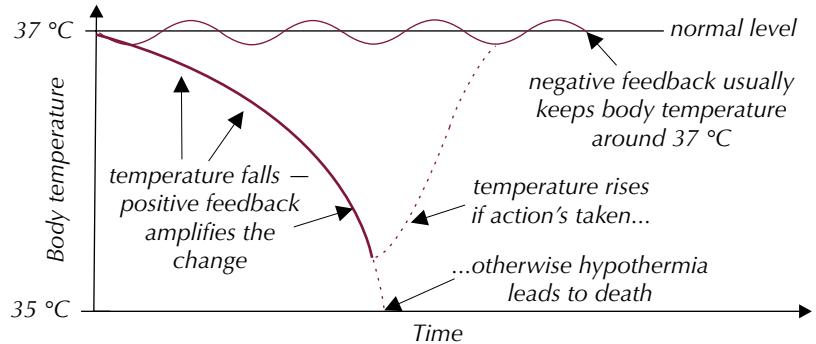


Figure 8: Positive feedback takes body temperature away from its normal level in people with hypothermia.

Practice Questions — Application

- Q1 The range of normal blood pH is between 7.35 and 7.45. Metabolic acidosis is a condition that occurs when blood pH falls below 7.35.
- Analysis of a patient's blood revealed that their hydrogen ion concentration $[H^+]$ was $5.50 \times 10^{-8} \text{ mol dm}^{-3}$. Use the formula $\text{pH} = -\log_{10} [H^+]$ to calculate the pH of the patient's blood. Use your answer to determine whether the patient could be suffering from metabolic acidosis.
 - Why is it important to maintain a blood pH in the normal range?

- Q2 Read the following two passages about control systems in the body:

Passage A

A high blood concentration of carbon dioxide lowers the pH of the blood. Chemoreceptors in the blood vessels detect this change and send signals to the brain to increase the respiration rate.

Passage B

When oestrogen concentration is high it stimulates the anterior pituitary gland to release LH. LH stimulates the ovaries to release more oestrogen.

For each passage, state whether it's an example of negative or positive feedback and explain your answer.

Practice Questions — Fact Recall

- What is homeostasis?
- Explain why it is important for the body to maintain its internal temperature within normal limits.
- Why is it important that a stable blood glucose concentration is maintained?
- Describe how positive feedback mechanisms differ from negative feedback mechanisms.

2. Control of Blood Glucose Concentration

Blood glucose concentration is under tight control by a hormonal system.

Glucose concentration in the blood

All cells need a constant energy supply to work — so blood glucose concentration must be carefully controlled. The concentration of glucose in the blood is normally around 90 mg per 100 cm³ of blood. It's monitored by cells in the **pancreas**. Blood glucose concentration rises after eating food containing carbohydrate. It falls after exercise, as more glucose is used in respiration to release energy.

Hormonal control of blood glucose concentration

The hormonal system controls blood glucose concentration using two hormones called **insulin** and **glucagon**. Like all hormones, insulin and glucagon are chemical messengers that travel in the blood to their target cells (effectors). They're both secreted by clusters of cells in the pancreas called the **islets of Langerhans**.

The islets of Langerhans contain **beta (β) cells** and **alpha (α) cells** (see Figure 1). β cells secrete insulin into the blood.

α cells secrete glucagon into the blood. Insulin and glucagon act on effectors, which respond to restore the blood glucose concentration to the normal level.

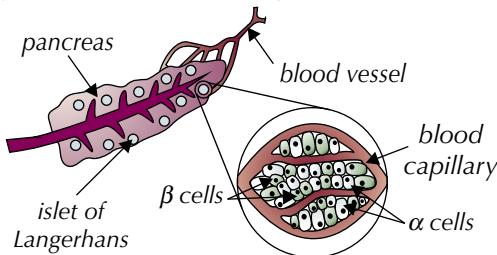


Figure 1: The location of α and β cells in the islets of Langerhans.

Insulin

Insulin lowers blood glucose concentration when it's too high. It binds to specific receptors on the cell membranes of muscle cells and liver cells (hepatocytes). It increases the permeability of muscle-cell membranes to glucose, so the cells take up more glucose. This involves increasing the number of channel proteins in the cell membranes (see page 361 for more).

Insulin also activates enzymes in muscle and liver cells that convert glucose into glycogen. The cells are able to store glycogen in their cytoplasm, as an energy source. The process of forming glycogen from glucose is called **glycogenesis** (see Figure 2). Insulin also increases the rate of respiration of glucose, especially in muscle cells.

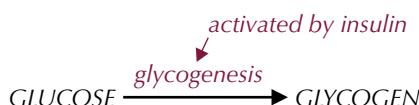


Figure 2: The process of glycogenesis.

Glucagon

Glucagon raises blood glucose concentration when it's too low. It binds to specific receptors on the cell membranes of liver cells and activates enzymes that break down glycogen into glucose. The process of breaking down glycogen is called **glycogenolysis**.

Learning Objectives:

- Know the factors that influence blood glucose concentration.
- Understand the role of the liver in glycogenesis, glycogenolysis and gluconeogenesis.
- Understand the action of insulin by attaching to receptors on the surfaces of target cells and activating enzymes involved in the conversion of glucose to glycogen.
- Understand the action of glucagon by attaching to receptors on the surfaces of target cells, activating enzymes involved in the conversion of glycogen to glucose and activating enzymes involved in the conversion of glycerol and amino acids into glucose.
- Understand the action of insulin in controlling the uptake of glucose by regulating the inclusion of channel proteins in the surface membranes of target cells.
- Understand the role of adrenaline by attaching to receptors on the surfaces of target cells and activating enzymes involved in the conversion of glycogen to glucose.
- Know the second messenger model of adrenaline and glucagon action, involving adenylate cyclase, cyclic AMP (cAMP) and protein kinase.

Specification Reference 3.6.4.2

Glucagon also activates enzymes that are involved in the formation of glucose from glycerol (a component of lipids) and amino acids. The process of forming glucose from non-carbohydrates is called **gluconeogenesis** (see Figure 3). Glucagon decreases the rate of respiration of glucose in cells.



Tip: Hormones (like insulin and glucagon) only bind to cells with specific receptors (target cells).

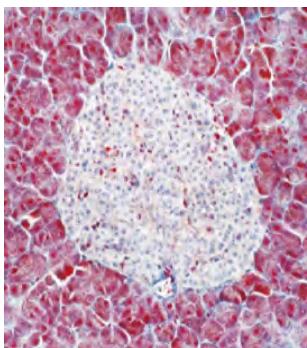


Figure 4: Islet of Langerhans (white) in the pancreas containing α cells and β cells.

Tip: ‘Genesis’ means ‘making’ — so glycogenesis means making glycogen.

Figure 3: The processes of glycogenolysis and gluconeogenesis.

Because they travel in the blood to their target cells, the responses produced by hormones are slower than those produced by nervous impulses (which are very quick — see page 315). It also means that responses to hormones can occur all over the body if their target cells are widespread, unlike nervous impulses that are localised to one area. Hormones are not broken down as quickly as neurotransmitters though, so their effects tend to last for longer.

Negative feedback mechanisms and glucose concentration

Negative feedback mechanisms keep blood glucose concentration normal.

Rise in blood glucose concentration

When the pancreas detects blood glucose concentration is too high, the β cells secrete insulin and the α cells stop secreting glucagon. Insulin then binds to receptors on liver and muscle cells (the effectors). The liver and muscle cells respond to decrease the blood glucose concentration, e.g. glycogenesis is activated (see previous page). Blood glucose concentration then returns to normal.

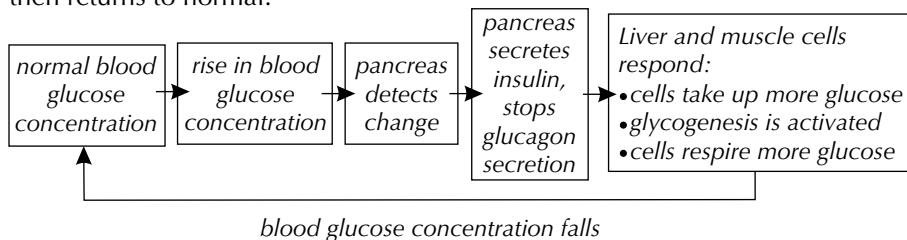


Figure 5: Negative feedback mechanism activated by a rise in blood glucose.

Tip: ‘Lysis’ means ‘splitting’ — so glycogenolysis means splitting glycogen.

Fall in blood glucose concentration

When the pancreas detects blood glucose is too low, the α cells secrete glucagon and the β cells stop secreting insulin. Glucagon then binds to receptors on liver cells (the effectors). The liver cells respond to increase the blood glucose concentration, e.g. glycogenolysis is activated (see previous page). Blood glucose concentration then returns to normal.

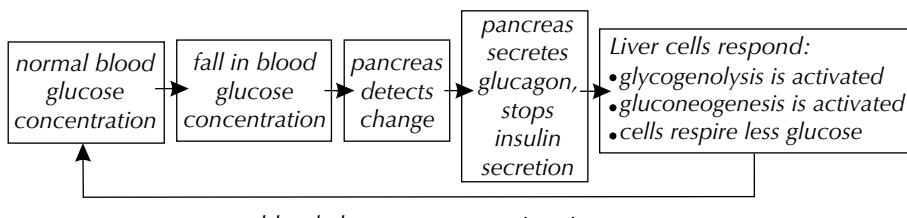


Figure 6: Negative feedback mechanism activated by a fall in blood glucose.

Glucose transporters

Glucose transporters are **channel proteins** which allow glucose to be transported across a cell membrane. Skeletal and cardiac muscle cells contain a glucose transporter called GLUT4. When insulin levels are low, GLUT4 is stored in vesicles in the cytoplasm of cells, but when insulin binds to receptors on the cell-surface membrane, it triggers the movement of GLUT4 to the membrane. Glucose can then be transported into the cell through the GLUT4 protein by facilitated diffusion.

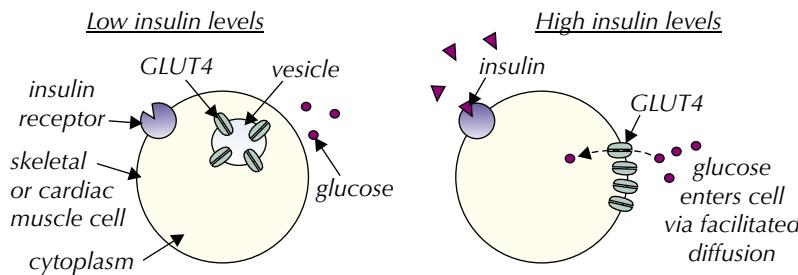


Figure 7: The effect of insulin on the availability of GLUT4.

Tip: Facilitated diffusion transports large or charged particles across a cell membrane down a concentration gradient (from a higher concentration to a lower concentration). It's a passive process so it doesn't require any energy.

Adrenaline

Adrenaline is a hormone that's secreted from your adrenal glands (found just above your kidneys). It's secreted when there's a low concentration of glucose in your blood, when you're stressed and when you're exercising. Adrenaline binds to receptors in the cell membrane of liver cells and does these things to increase blood glucose concentration:

- It activates glycogenolysis (the breakdown of glycogen to glucose).
- It inhibits glycogenesis (the synthesis of glycogen from glucose).

It also activates glucagon secretion and inhibits insulin secretion, which increases glucose concentration. Adrenaline gets the body ready for action by making more glucose available for muscles to respire.

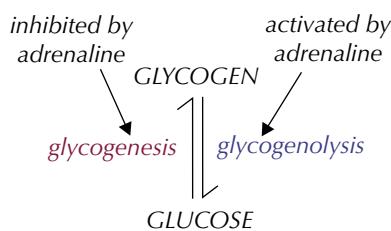


Figure 8: The effect of adrenaline on glycogenesis and glycogenolysis.

Second messengers

Both adrenaline and glucagon can activate glycogenolysis inside a cell even though they bind to receptors on the outside of the cell. They do this by the **second messenger model** — the binding of the hormone to cell receptors activates an enzyme on the inside of the cell membrane, which then produces a chemical known as a second messenger. The second messenger activates other enzymes in the cell to bring about a response.

The receptors for adrenaline and glucagon have specific tertiary structures that make them complementary in shape to their respective hormones. To activate glycogenolysis, adrenaline and glucagon bind to their receptors and activate an enzyme called **adenylate cyclase**. Activated adenylate cyclase converts ATP into a chemical called **cyclic AMP (cAMP)**, which is a second messenger. cAMP activates an enzyme called **protein kinase A**. Protein kinase A activates a cascade (a chain of reactions) that breaks down glycogen into glucose (glycogenolysis) — see Figure 9 on the next page.

Tip: Remember, adrenaline and glucagon bind to receptors on the cell membranes of liver cells.

Tip: Adenylate cyclase is also known as adenylyl cyclase.

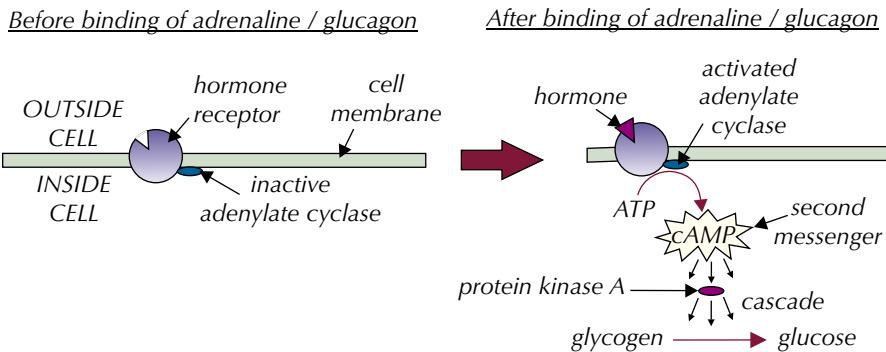


Figure 9: Second messenger model of adrenaline and glucagon action.

Exam Tip

There's lots of similar sounding words in this section so you need to make sure you get your spelling spot on in the exam, e.g. if you write 'glycogen' the examiner won't know whether you mean glucagon or glycogen so you won't get the marks.

Summary of blood glucose control

| Process | Converts | Activated by | Inhibited by |
|-----------------|-----------------------------------|-------------------------|--------------|
| Glycogenesis | Glucose to glycogen | Insulin | Adrenaline |
| Glycogenolysis | Glycogen to glucose | Glucagon and adrenaline | — |
| Gluconeogenesis | Glycerol / amino acids to glucose | Glucagon | — |

Practice Questions — Application

- Q1 Describe how a person's blood glucose concentration will change after eating a big bowl of pasta and explain how their body returns it back to normal.
- Q2 Von Gierke's disease is a glycogen storage disease. It's caused by an enzyme deficiency, which means the processes of glycogenolysis and gluconeogenesis can't work properly. Explain why someone with von Gierke's disease might suffer from hypoglycaemia if they don't eat regularly.

Tip: Hypoglycaemia means low blood glucose concentration.

Practice Questions — Fact Recall

- Q1 Where are insulin and glucagon secreted from?
- Q2 Give three ways in which insulin reduces blood glucose concentration.
- Q3 Name the process that converts glucose to glycogen.
- Q4 Name and describe two processes activated by glucagon.
- Q5 Describe the negative feedback mechanism that is activated by a fall in blood glucose concentration.
- Q6 Describe how insulin regulates the uptake of glucose into skeletal and cardiac muscle cells.
- Q7 Explain how adrenaline brings about glycogenolysis when blood glucose concentration is low.

3. Diabetes and Blood Glucose Concentration

Diabetes is an example of when homeostasis goes awry...

What is diabetes?

Diabetes mellitus is a condition where blood glucose concentration can't be controlled properly. There are two types:

Type I

In Type I diabetes, the immune system attacks the β cells in the islets of Langerhans so they can't produce any insulin. After eating, the blood glucose level rises and stays high — this is called hyperglycaemia and can result in death if left untreated. The kidneys can't reabsorb all this glucose, so some of it's excreted in the urine. Type I diabetes is treated with insulin therapy. Most people with Type I diabetes need regular insulin injections throughout the day, but some people use an insulin pump to deliver insulin continuously instead. Insulin therapy has to be carefully controlled because too much can produce a dangerous drop in blood glucose levels — this is called hypoglycaemia. Eating regularly and controlling simple carbohydrate intake (intake of sugars) helps to avoid a sudden rise in glucose.

No one knows exactly what causes the immune system to attack the β cells and cause Type I diabetes. Scientists have found that some people have a genetic predisposition to developing Type I diabetes. They also think that the disease may be triggered by a viral infection.

Type II

Type II diabetes is usually acquired later in life than Type I. It is often linked with obesity and is more likely in people with a family history of the condition. Other risk factors include lack of exercise, age and poor diet. Type II diabetes occurs when the β cells don't produce enough insulin or when the body's cells don't respond properly to insulin. Cells don't respond properly because the insulin receptors on their membranes don't work properly, so the cells don't take up enough glucose. This means the blood glucose concentration is higher than normal. It can be treated by eating a healthy, balanced diet, losing weight (if necessary) and regular exercise. Glucose-lowering medication can be taken if diet and exercise can't control it. Eventually, insulin injections may be needed.

Responses to Type II diabetes

Type II diabetes is becoming increasingly common in the UK. This has been linked to increasing levels of obesity, a move towards more unhealthy diets and low levels of physical activity. Type II diabetes can cause additional health problems, including visual impairment and kidney failure, so health advisors are understandably keen to educate people about the risks and reduce the incidence of the disease. Some people also think the food industry has a role to play in tackling the problem.

You need to understand the various responses to the increase in Type II diabetes and be able to evaluate them.

Response of health advisors

To reduce the risk of developing Type II diabetes, health advisors recommend that people eat a diet that's low in fat, sugar and salt, with plenty of whole grains, fruit and vegetables, take regular exercise and lose weight if necessary.

Learning Objectives:

- Understand the causes of Types I and II diabetes and their control by insulin and/or manipulation of the diet.
- Be able to evaluate the positions of health advisers and the food industry in relation to the increased incidence of Type II diabetes.
- Be able to produce a dilution series of a glucose solution and use colorimetric techniques to produce a calibration curve with which to identify the concentration of glucose in an unknown 'urine' sample (Required Practical 11).

Specification Reference 3.6.4.2

Tip: Simple carbohydrates are more easily broken down to glucose, which is then absorbed by the digestive system, than complex carbohydrates.

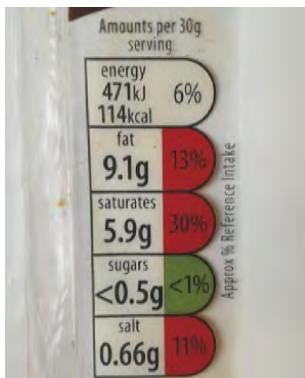


Figure 1: Clearer labelling can help people make healthier choices.

Tip: Some people believe that diet varieties are not as good for health as they are claimed to be, e.g. there is some evidence to suggest that artificial sweeteners are linked to weight gain.

Tip: Don't worry, it won't be real urine! You'll be given a fake sample by your teacher.

Tip: Make sure you do a risk assessment before starting this experiment. The Benedict's test requires you to heat test tubes in a water bath, so take care not to touch them when they're hot. You should also wear safety goggles when working with Benedict's reagent.

Tip: When you're testing for a low concentration of glucose in a solution, quantitative Benedict's reagent can give a more accurate result than normal Benedict's reagent.

Campaigns like the NHS's 'Change4Life', aim to educate people on how to have a healthier diet and lifestyle, and so reduce their risk of developing conditions like Type II diabetes. Health advisors have also challenged the food industry to reduce the advertising of junk food (particularly to children), to improve the nutritional value of their products, and to use clearer labelling on products — allowing consumers to make healthier choices about what to buy.

Response of food companies

In response to criticism, some food companies have attempted to make their products more healthy. For example, by using sugar alternatives to sweeten food and drinks, and by reducing the sugar, fat and salt content of products.

However, there is pressure on companies to increase profits — they're reluctant to spend money developing new, healthier alternatives if the more unhealthy products are still popular and generate lots of profit. They say that the industry will only respond fully in the long term, as public perception about healthy eating changes.

Glucose in urine

If it is suspected that a person has diabetes, a doctor may request that a sample of their urine is tested for glucose. Normally, the concentration of glucose in urine is very low — between 0 and 0.8 mM. Higher concentrations than this may indicate diabetes (although a blood test would be needed to confirm it).

Determining the concentration of a glucose solution

You need to be able to determine the concentration of glucose in a 'urine' sample, using **colorimetry**.

REQUIRED
PRACTICAL **11**

To do this you can use a quantitative Benedict's test. Quantitative Benedict's reagent is different to normal Benedict's reagent. When heated with glucose, the initial blue colour is lost, but a brick-red precipitate is not produced. You can use a colorimeter to measure the light absorbance of the solution after the quantitative Benedict's test has been carried out. The higher the concentration of glucose, the more blue colour will be lost (i.e. the paler the solution will become), decreasing the absorbance of the solution.

Making serial dilutions

Initially you need to make up several glucose solutions of different, known concentrations. You can do this using a serial dilution technique:

Example

This is how you'd make five serial dilutions with a dilution factor of 2, starting with an initial glucose concentration of 4 mM:

1. Line up five test tubes in a rack.
2. Add 10 cm³ of the initial 4 mM glucose solution to the first test tube and 5 cm³ of distilled water to the other four test tubes.
3. Then, using a pipette, draw 5 cm³ of the solution from the first test tube, add it to the distilled water in the second test tube and mix the solution thoroughly. You now have 10 cm³ of solution that's half as concentrated as the solution in the first test tube (it's 2 mM).
4. Repeat this process three more times to create solutions of 1 mM, 0.5 mM and 0.25 mM (see Figure 2 — next page).

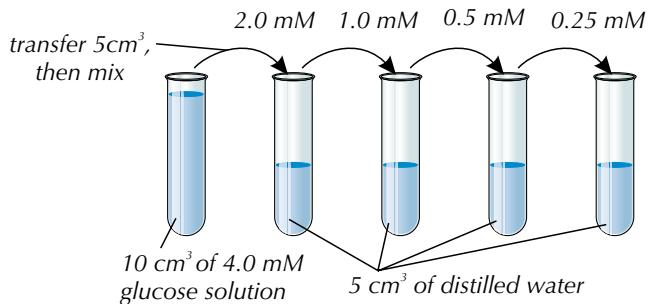


Figure 2: How to make serial dilutions.

Measuring the absorbance of glucose solutions

Once you've got your glucose solutions, you need to find out the absorbance of each one. Here's how:

1. Make sure you are using equal volumes of each of the solutions. You should also set up a test tube containing only pure water, which will act as a negative control.
2. Add the same amount of quantitative Benedict's reagent to each test tube and stir gently to mix.
3. Heat the test tubes for 4-5 minutes in a water bath that's been brought to the boil. Make sure you heat them all for the same amount of time.
4. Carefully remove the test tubes from the water bath and leave to cool.
5. Next, use a colorimeter to measure the absorbance of the solution in each test tube (see below).

Tip: There's no glucose in the negative control, so there's nothing for the Benedict's reagent to react with. This means the solution will remain blue — this should give you the highest absorbance value in the experiment.

Using a colorimeter

A colorimeter is a device that measures absorbance (the amount of light absorbed by a solution). You've come across one before, but here's a quick recap:

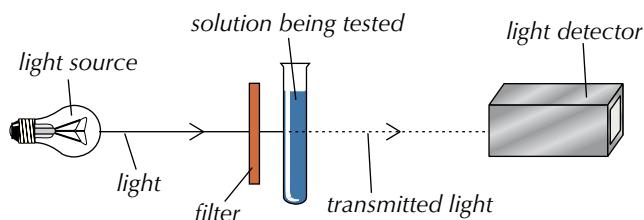


Figure 3: A diagram showing how a colorimeter works.

For this experiment, you will need to set up the colorimeter with a red filter (or a wavelength of 635 nm).

After turning on the machine and allowing it time to stabilise, calibrate it to zero using a cuvette of distilled water. Then use a pipette to transfer a sample of the solution you would like to test into a clean cuvette and measure the absorbance of the solution. For each solution you test, use a clean pipette and cuvette.

Tip: Don't forget to zero the colorimeter between each reading.

Making and using a calibration curve

Tip: When drawing a calibration curve, the curve should go through or near as many points as possible.

Tip: A calibration curve doesn't actually have to be a 'curve' — it can be a straight line (but you still call it a calibration curve).

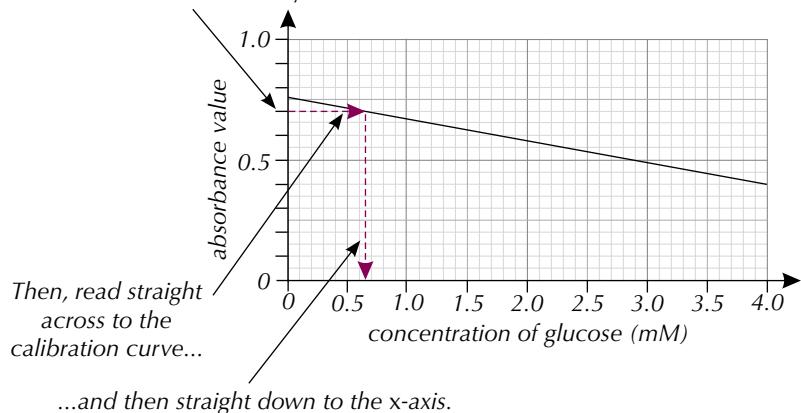
Once you've measured the absorbance of each glucose solution, you can make a calibration curve. To do this, plot a graph of your results showing absorbance (on the y-axis) against glucose concentration (on the x-axis). Draw a smooth line/curve of best fit through your data points to create a calibration curve.

Then you can test the unknown solution (the 'urine' sample) in the same way as the known concentrations — i.e. use the same volume of solution and quantitative Benedict's reagent, and heat the solution for the same amount of time. Once you've measured the absorbance of the unknown solution you can use the calibration curve to find its concentration.

Example — Maths Skills

Use the calibration curve below to find the glucose concentration of an unknown glucose solution with an absorbance value of 0.7.

Firstly, find the absorbance value of the unknown solution on the y-axis.



So here, an unknown solution with an absorbance value of 0.7 has a glucose concentration of **0.65 mM**.

Practice Questions — Application

Q1 The table below shows the blood glucose concentration of a person with Type I diabetes taken at various times throughout the day. The person controls her diabetes with insulin injections after meals and aims to keep her blood glucose concentration between 4 and 7 mM.

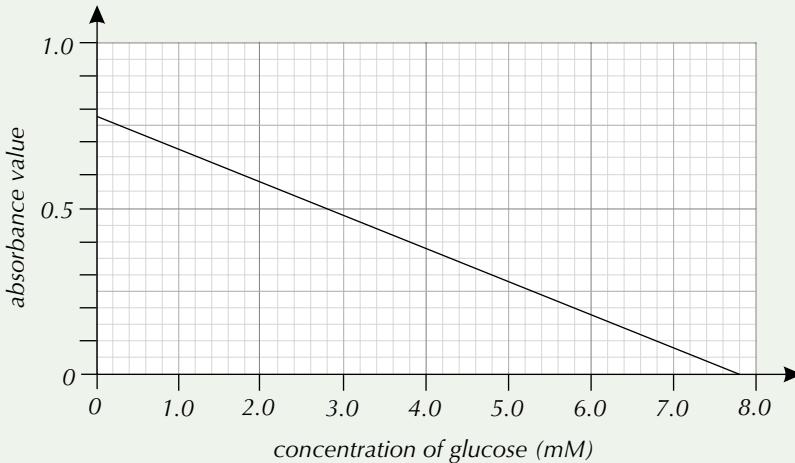
| | Blood glucose concentration (mM) | | |
|-------|----------------------------------|----------------------|---------------------|
| | Before lunch | One hour after lunch | Before evening meal |
| Day 1 | 4.2 | 8.7 | 5.0 |
| Day 2 | 3.5 | 7.3 | 6.7 |

- On which day do you think the person ate a lunch with the highest proportion of simple carbohydrates? Explain your answer.
- Give one assumption that you have made in your answer to a) about the insulin injections.

Tip: There is a time delay between an insulin injection and the reduction of blood glucose concentration. This is because the insulin first needs to travel in the blood to its target cells.

Q2 A student used a colorimeter to measure the absorbance of known concentrations of glucose, after doing a quantitative Benedict's test on each solution, and used the results to draw the calibration curve below. The student also carried out the test on a 'urine' sample with an unknown glucose concentration.

- a) The 'urine' sample gave an absorbance reading of 0.4. Using the calibration curve, find the glucose concentration of the sample.



- b) The glucose concentration in urine is normally between 0 and 0.8 mM. What range of absorbance values would the student expect normal urine samples to give in this investigation?

Exam Tip

Remember to be really careful when reading values from a graph in the exams — you don't want to throw away marks because you've rushed and misread the scale.

Practice Questions — Fact Recall

- Q1 What causes Type I diabetes?
- Q2 Give two risk factors associated with Type II diabetes.
- Q3 Describe how Type II diabetes can be controlled.
- Q4 Give three actions that health advisors think the food industry needs to do to reduce the risk of people developing Type II diabetes.
- Q5 Describe how you could use serial dilutions of a glucose solution of known concentration to find the glucose concentration of a urine sample.

Learning Objective:

- Recall the structure of the nephron and its role in:
 - the formation of glomerular filtrate,
 - reabsorption of glucose and water by the proximal convoluted tubule,
 - reabsorption of water by the distal convoluted tubule and collecting ducts.

Specification Reference 3.6.4.3

Tip: The kidneys also regulate the body's water content — there's more about this on pages 371-373.

4. The Kidneys

One of the main functions of the kidneys is to filter waste products out of the blood and reabsorb useful solutes (e.g. glucose).

Excretion of waste products

Blood enters the kidney through the renal artery and then passes through capillaries in the cortex (outer layer) of the kidneys. As the blood passes through capillaries in the cortex, substances are filtered out of the blood and into long tubules that surround the capillaries. This process is called **ultrafiltration** (see below). Useful substances, such as glucose and the right amount of water, are then reabsorbed back into the blood. This process is called **selective reabsorption**. The remaining unwanted substances pass along to the bladder and are excreted as urine.

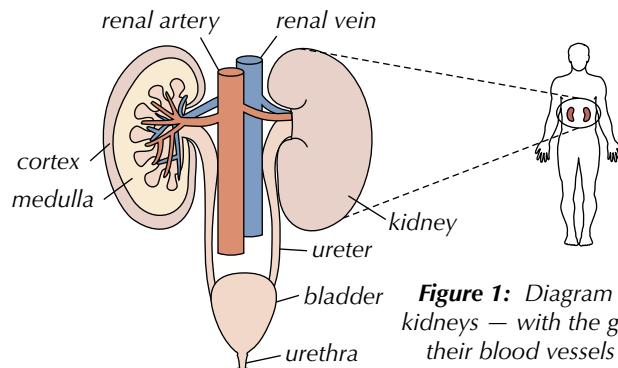


Figure 1: Diagram to show the location of the kidneys — with the gross structure of the kidneys, their blood vessels and the bladder enlarged.

The nephrons

The long tubules along with the bundles of capillaries where the blood is filtered are called nephrons — there are around one million nephrons in each kidney. You need to learn the structure of a nephron (see Figure 3).

Tip: 'Renal' means anything to do with the kidney.

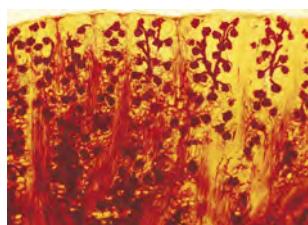


Figure 2: Light micrograph of a section through the cortex, showing the glomeruli (tiny balls) and the vessels that supply them.

Ultrafiltration

Blood from the renal artery enters smaller arterioles in the cortex of the kidney. Each arteriole splits into a structure called a glomerulus (plural, glomeruli) — a bundle of capillaries looped inside a hollow ball called a Bowman's capsule (see Figure 3). This is where ultrafiltration takes place.

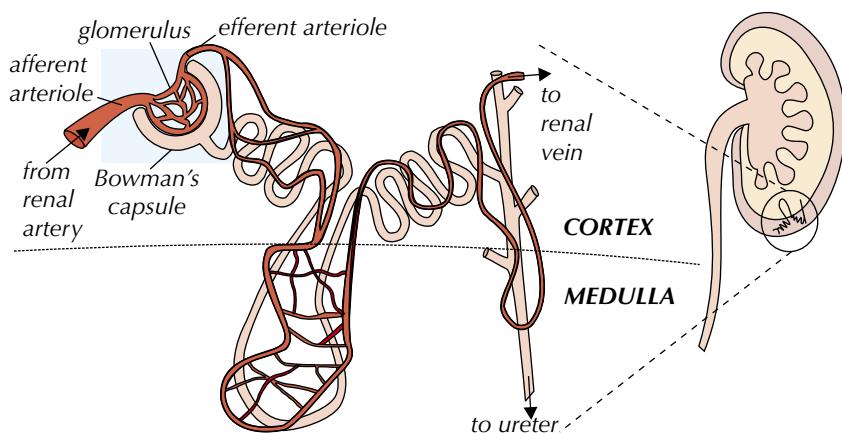
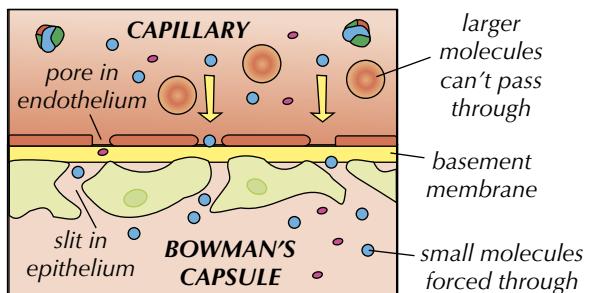


Figure 3: The location and structure of one nephron. Ultrafiltration takes place in the glomerulus and Bowman's capsule (highlighted in blue).

The arteriole that takes blood into each glomerulus is called the afferent arteriole, and the arteriole that takes the filtered blood away from the glomerulus is called the efferent arteriole (see Figure 3 on the previous page). The efferent arteriole is smaller in diameter than the afferent arteriole, so the blood in the glomerulus is under high pressure. The high pressure forces liquid and small molecules in the blood out of the capillary and into the Bowman's capsule.

The liquid and small molecules pass through three layers to get into the Bowman's capsule and enter the nephron tubules — the capillary endothelium, a membrane (called the basement membrane) and the epithelium of the Bowman's capsule (see Figure 4).

Figure 4: Diagram to show the three layers separating the glomerular capillary and the Bowman's capsule.



Tip: The cells that make up the epithelium of the Bowman's capsule are called podocytes.

Larger molecules like proteins and blood cells can't pass through so stay in the blood. The substances that enter the Bowman's capsule are known as the **glomerular filtrate**. The glomerular filtrate passes along the rest of the nephron and useful substances are reabsorbed along the way — see below. Finally, the filtrate flows through the collecting duct and passes out of the kidney along the ureter.

Tip: The glomerular filtrate can also be called the tubular fluid.

Selective reabsorption

Selective reabsorption of useful substances takes place as the glomerular filtrate flows along the proximal convoluted tubule (PCT), through the loop of Henle, and along the distal convoluted tubule (DCT) — see Figure 5. Useful substances leave the tubules of the nephrons and enter the capillary network that's wrapped around them.

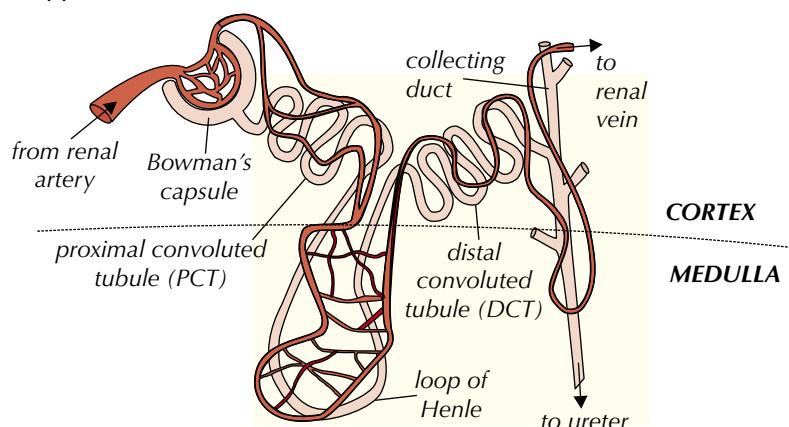


Figure 5: Diagram to show the structure of one nephron.
Selective reabsorption takes place in the areas highlighted in yellow.

The epithelium of the wall of the PCT has microvilli to provide a large surface area for the reabsorption of useful materials from the glomerular filtrate (in the tubules) into the blood (in the capillaries) — see Figure 7 on the next page. Useful solutes, like glucose, are reabsorbed along the PCT by **active transport** and **facilitated diffusion**.

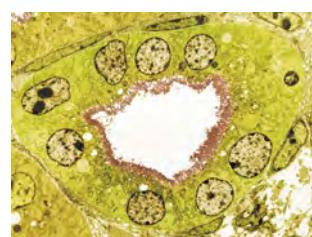


Figure 6: Electron micrograph of a cross-section through the proximal convoluted tubule (PCT). Microvilli (shown in reddish-brown) line the inside of the tubule, increasing the surface area for reabsorption.

Tip: Remember, water potential describes the tendency of water to move from one area to another. Water will move from an area of higher water potential to an area of lower water potential — it moves down the water potential gradient.

Tip: Urea is a waste product produced from the breakdown of amino acids in the liver.

Tip: The volume of water in urine varies depending on how much you've drunk (see pages 371-373).

Water enters the blood by **osmosis** because the water potential of the blood is lower than that of the filtrate. Water is reabsorbed from the PCT, loop of Henle, DCT and the collecting duct (see next page). The filtrate that remains is urine, which passes along the ureter to the bladder.

Urine

Urine is usually made up of water and dissolved salts, urea and other substances such as hormones and excess vitamins. Urine doesn't usually contain proteins or blood cells as they're too big to be filtered out of the blood. Glucose is actively reabsorbed back into the blood (see previous page), so it's not usually found in the urine either.

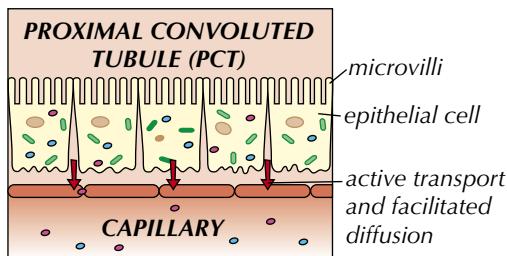
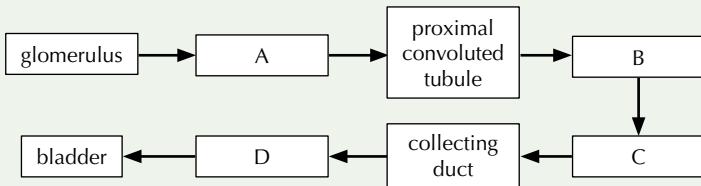


Figure 7: Epithelial wall of the proximal convoluted tubule (PCT).

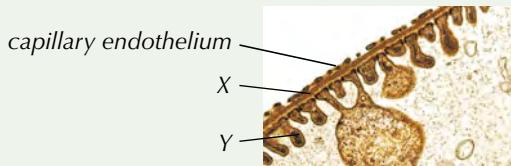
Practice Questions — Application

Q1 The kidneys filter the blood in order to produce urine.

The flow diagram below shows the sequence of urine production. Name the missing structures, A to D.



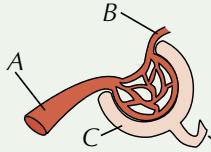
Q2 The diagram below shows an electron micrograph of a cross-section of the barrier between the Bowman's capsule and the blood supply.



- Name the structures labelled X and Y.
- Hereditary nephrotic syndrome is an inherited disease which affects the structure of the barrier shown above, resulting in the presence of large amounts of protein in the urine (proteinuria). Suggest why hereditary nephrotic syndrome causes proteinuria.

Practice Questions — Fact Recall

Q1 The diagram below shows a glomerulus and surrounding structures.



- Name blood vessel A.
- Name the structure labelled C.
- Vessel A has a larger diameter than vessel B. Explain why this is important in the process of ultrafiltration.

Q2 Name two substances that are reabsorbed in the proximal convoluted tubule.

5. Controlling Blood Water Potential

After the last few pages you might feel like you know all there is to know about nephrons — but there's more. Now it's time to see how they're involved in controlling the water potential of the blood.

Regulation of water content

Water is essential to keep the body functioning, so the amount of water in the blood (and so the water potential of the blood) needs to be kept constant. Mammals excrete urea (and other waste products) in solution, which means water is lost during excretion. Water is also lost in sweat. The kidneys regulate the water potential of the blood (and urine), so the body has just the right amount of water — this is called **osmoregulation**:

- If the water potential of the blood is too low (the body is dehydrated), more water is reabsorbed by osmosis into the blood from the tubules of the nephrons. This means the urine is more concentrated, so less water is lost during excretion.
- If the water potential of the blood is too high (the body is too hydrated), less water is reabsorbed by osmosis into the blood from the tubules of the nephrons. This means the urine is more dilute, so more water is lost during excretion.

Learning Objectives:

- Be able to explain osmoregulation as control of the water potential of the blood.
- Recall the role of the nephron in maintaining a gradient of sodium ions in the medulla by the loop of Henle.
- Recall the roles of the hypothalamus, posterior pituitary and antidiuretic hormone (ADH) in osmoregulation.

Specification Reference 3.6.4.3

Tip: For more on reabsorption in the nephrons, see pages 369–370.

Water is reabsorbed into the blood along almost all of the nephron (see previous page), but regulation of water potential mainly takes place in the loop of Henle, DCT and collecting duct (see below). The volume of water reabsorbed by the DCT and collecting duct is controlled by hormones (see next page).

The loop of Henle

The loop of Henle is located in the medulla (inner layer) of the kidneys. It's made up of two 'limbs' — the descending limb and the ascending limb. The limbs control the movement of sodium ions so that water can be reabsorbed by the blood.

Tip: Figure 1 is explained in detail on the next page.

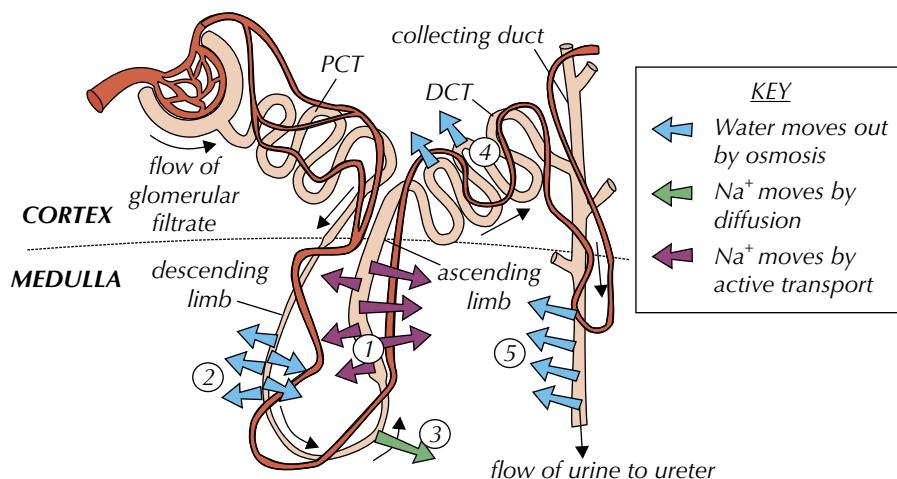


Figure 1: The movement of water and Na⁺ in the loop of Henle, DCT and collecting duct.

Tip: Na⁺ is a sodium ion. These ions help establish the water potential that drives the reabsorption of water from the glomerular filtrate back into the blood.

Here's how the system works:

1. Near the top of the ascending limb, Na^+ ions are actively pumped out into the medulla. The ascending limb is impermeable to water, so the water stays inside the tubule. This creates a low water potential in the medulla, because there's a high concentration of ions.
2. Because there's a lower water potential in the medulla than in the descending limb, water moves out of the descending limb (which is permeable to water) into the medulla by osmosis. This makes the glomerular filtrate more concentrated (the ions can't diffuse out — the descending limb isn't permeable to them). The water in the medulla is reabsorbed into the blood through the capillary network.
3. Near the bottom of the ascending limb Na^+ ions diffuse out into the medulla, further lowering the water potential in the medulla. (The ascending limb is impermeable to water, so it stays in the tubule.)
4. Water moves out of the distal convoluted tubules (DCT) by osmosis and is reabsorbed into the blood.
5. The first three stages massively increase the ion concentration in the medulla, which lowers the water potential. This causes water to move out of the collecting duct by osmosis. As before, the water in the medulla is reabsorbed into the blood through the capillary network.

The volume of water reabsorbed into the capillaries is controlled by changing the permeability of the DCT and the collecting duct (see below).

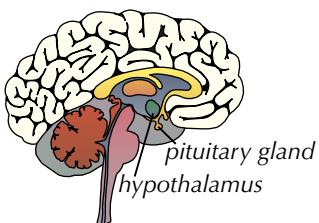


Figure 2: Location of the hypothalamus and the pituitary gland in the brain.

Tip: Diuresis is when lots of dilute urine is produced. Antidiuretic hormone is so called because it causes a small amount of concentrated urine to be produced (the opposite of diuresis).

Antidiuretic hormone (ADH)

The water potential of the blood is monitored by cells called **osmoreceptors** in a part of the brain called the **hypothalamus**. When the water potential of the blood decreases, water will move out of the osmoreceptor cells by osmosis. This causes the cells to decrease in volume. This sends a signal to other cells in the hypothalamus, which send a signal to the **posterior pituitary gland**. This causes the posterior pituitary to release a hormone called antidiuretic hormone (ADH) into the blood.

ADH molecules bind to receptors on the plasma membranes of cells in the DCT and the collecting duct. When this happens, protein channels called aquaporins are inserted into the plasma membrane. These channels allow water to pass through via osmosis, making the walls of the DCT and collecting duct more permeable to water. This means more water is reabsorbed from these tubules into the medulla and into the blood by osmosis. A small amount of concentrated urine is produced, which means less water is lost from the body.

ADH changes the water content of the blood when it's too low or too high:

Dehydration — blood water content is too low

Dehydration is what happens when you lose water, e.g. by sweating during exercise, so the water content of the blood needs to be increased:

- The water content of the blood drops, so its water potential drops.
- This is detected by osmoreceptors in the hypothalamus.
- The posterior pituitary gland is stimulated to release more ADH into the blood.
- More ADH means that the DCT and collecting duct are more permeable, so more water is reabsorbed into the blood by osmosis.

- A small amount of highly concentrated urine is produced and less water is lost.

Hydration — blood water content is too high

If you're hydrated, you've taken in lots of water, so the water content of the blood needs to be reduced:

- The water content of the blood rises, so its water potential rises.
- This is detected by the osmoreceptors in the hypothalamus.
- The posterior pituitary gland releases less ADH into the blood.
- Less ADH means that the DCT and collecting duct are less permeable, so less water is reabsorbed into the blood by osmosis.
- A large amount of dilute urine is produced and more water is lost.

Tip: Like many hormones, ADH is a protein. Once it's had its effect, it travels in the bloodstream to the liver where it's broken down.

Practice Questions — Application

- Q1 A runner is dehydrated whilst running on a hot, sunny day. He left his drink at home and is producing a lot of sweat during his run.
- Why is the runner dehydrated?
 - How does the runner's body detect that he is dehydrated?
 - The runner's posterior pituitary gland releases antidiuretic hormone (ADH). Explain what effect ADH has on the distal convoluted tubule and the collecting duct of the runner's kidneys.
 - When he returns home, he rehydrates by drinking a sports drink containing sodium ions. Explain how the presence of these ions helps the runner's kidneys to conserve water.
- Q2 Exercise-associated hyponatremia (EAH) is a condition experienced by some athletes who drink excessive amounts of fluid when competing in endurance events like marathons. The condition affects the balance of fluid in cells and is potentially fatal if it affects the brain cells.
- Explain what normally happens when a person consumes too much fluid.
 - Athletes who experience EAH are often unable to suppress their ADH production. Explain why this can cause problems if they have consumed too much fluid.
- Q3 The fennec fox lives in a hot, dry environment. It has evolved a long loop of Henle to help it survive in this environment.
- Suggest and explain the advantage that having a longer loop of Henle gives the fennec fox in its environment.
 - Frogs and toads don't have any loops of Henle. Suggest why this is the case.

Practice Questions — Fact Recall

- What is osmoregulation?
- Name the layer of the kidney in which the loop of Henle is located.
- Which limb of the loop of Henle is impermeable to water?
- Which part of the brain monitors the water potential of the blood?

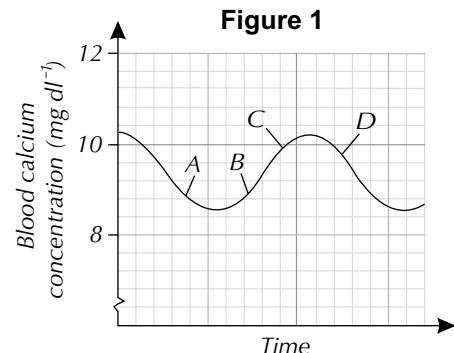
Section Summary

Make sure you know...

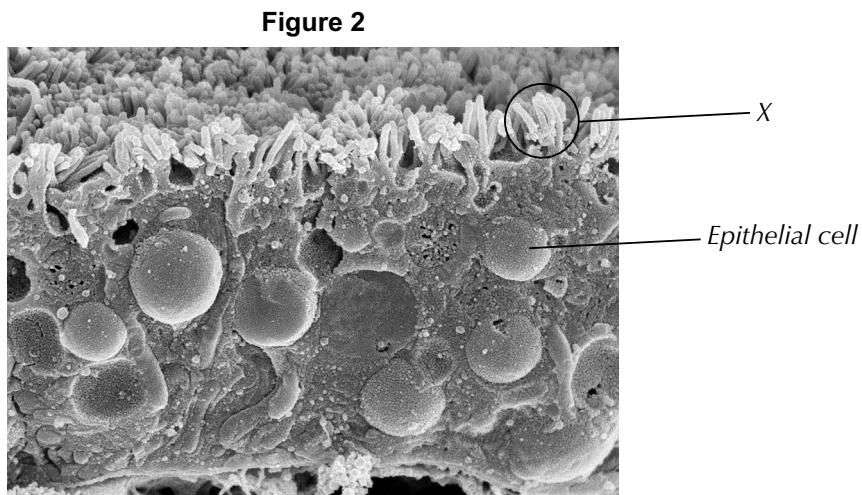
- That homeostasis in mammals involves physiological control systems that maintain a stable internal environment (within certain limits).
- That it's important to maintain a stable core body temperature and stable blood pH to provide the optimum conditions for enzymes to work.
- That it's important to maintain a stable blood glucose concentration to prevent the water potential of the blood becoming too low (if blood glucose concentration becomes too high) and to ensure there's enough glucose available for respiration.
- That negative feedback mechanisms return systems in the body back to their normal level.
- That multiple separate negative feedback mechanisms give more control over systems in the body.
- That positive feedback amplifies a change and is often involved in the breakdown of control systems.
- How to interpret information relating to positive and negative feedback.
- That diet and exercise affect blood glucose concentration.
- That glycogenesis is the conversion of glucose to glycogen, glycogenolysis is the conversion of glycogen to glucose, and gluconeogenesis is the conversion of glycerol or amino acids to glucose, and that all of these processes can occur in liver cells.
- That insulin lowers blood glucose level when it's too high by binding to receptors on liver and muscle cells and activating enzymes involved in glycogenesis, causing the cells to take up more glucose and causing the cells to respire more glucose.
- That glucagon raises blood glucose level when it's too low by binding to receptors on liver cells and activating enzymes involved in glycogenolysis and gluconeogenesis, and causing the cells to respire less glucose.
- That insulin increases the uptake of glucose by skeletal and cardiac muscle cells by increasing the amount of glucose transporters (channel proteins) in the cell membranes of those cells.
- The second messenger model of adrenaline and glucagon action — when adrenaline and glucagon bind to specific receptors on liver-cell membranes it activates an enzyme called adenylate cyclase, which then converts ATP into a second messenger called cyclic AMP (cAMP). This then activates an enzyme called protein kinase A, which activates a cascade inside the cell resulting in glycogenolysis.
- That Type I diabetes is caused when the immune system attacks the β cells in the islets of Langerhans meaning that no insulin is produced by the pancreas, and that Type II diabetes is caused when the β cells don't produce enough insulin or body cells no longer respond properly to it.
- That Type I diabetes is controlled by insulin therapy and Type II diabetes can often be controlled by eating a healthy, balanced diet, losing weight (if necessary), and regular exercise.
- The positions of health advisors and the food industry on the increasing incidence of Type II diabetes.
- How to use a colorimeter to determine the glucose concentration of a 'urine' sample, including how to make serial dilutions of a solution with a known concentration of glucose and use the absorbance values of the dilutions to produce a calibration curve (Required Practical 11).
- The structure of a nephron.
- That glomerular filtrate is the liquid and small molecules that pass into the Bowman's capsule following ultrafiltration of the blood entering the nephron.
- That glucose is reabsorbed into the blood from the proximal convoluted tubule and that water is reabsorbed from the proximal convoluted tubule, distal convoluted tubule and collecting ducts.
- That osmoregulation is the control of the water potential of the blood.
- How a gradient of sodium ions is maintained in the medulla by the loop of Henle.
- That the water content of the blood is monitored by osmoreceptors in the hypothalamus, and how the release of antidiuretic hormone (ADH) from the posterior pituitary gland is used to control the reabsorption of water in the nephrons.

Exam-style Questions

- 1 When low blood calcium concentration is detected, the secretion of parathyroid hormone (PTH) from the parathyroid gland is stimulated. When high blood calcium concentration is detected, the secretion of the hormone calcitonin, from the thyroid gland, is stimulated. These two hormones work via negative feedback mechanisms to control the blood calcium concentration. Their effects are shown in **Figure 1**.



- 1.1 Suggest an explanation for the shape of the graph between points A and D. (4 marks)
- 1.2 Why is it beneficial to have both PTH and calcitonin controlling the concentration of calcium in the blood? (2 marks)
- 1.3 Suggest what could happen to the blood calcium concentration of someone who has had a parathyroid gland removed. Explain your answer. (2 marks)
- 2 **Figure 2** is an electron micrograph showing a section through the proximal convoluted tubule of the kidney.



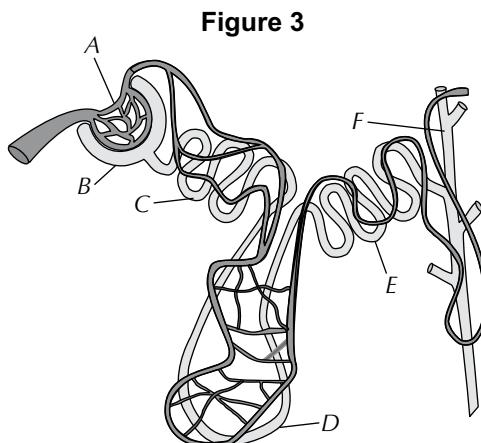
- 2.1 Outline what happens to glomerular filtrate in the proximal convoluted tubule. (1 mark)
- 2.2 Name the structure labelled X on **Figure 2** and explain how this structure helps the epithelial cells of the proximal convoluted tubule to carry out their function. (2 marks)

Figure 3 shows a nephron.

- 2.3 What name is given to structure **A**?
(1 mark)

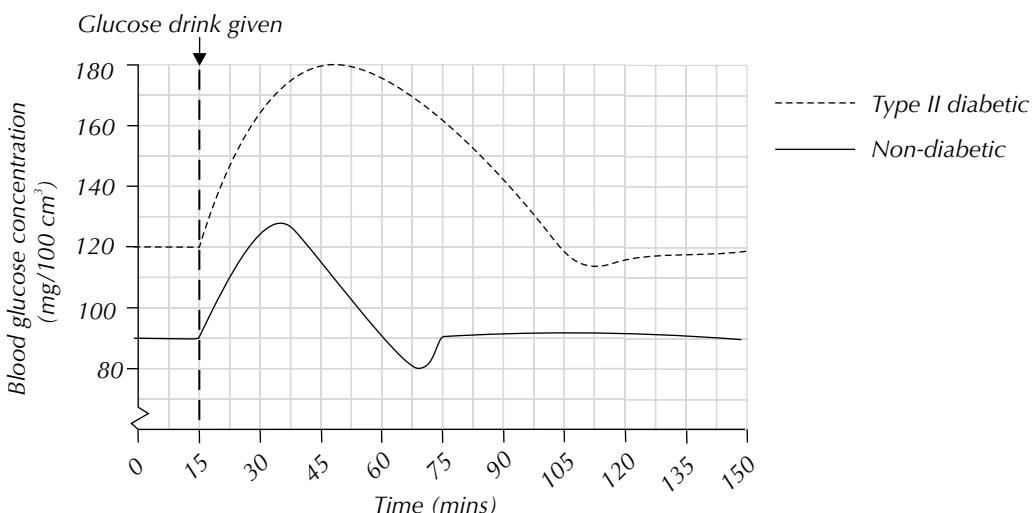
- 2.4 Which **two** letters (**A** to **F**) indicate the locations where antidiuretic hormone (ADH) acts?
(1 mark)

- 2.5 Name structure **D** and describe its main function in the process of reabsorption.
(2 marks)



- 3 In an experiment, the blood glucose concentrations of a Type II diabetic and a non-diabetic were recorded at regular intervals in a 150 minute time period. 15 minutes into the experiment a glucose drink was given. The normal range for blood glucose concentration in a healthy individual is between 82 and 110 mg/100 cm³. The results of the experiment are shown in **Figure 4**.

Figure 4



- 3.1 Describe how cells in the non-diabetic's islets of Langerhans are responding between 65 and 75 minutes.
(2 marks)
- 3.2 Explain why the Type II diabetic's blood glucose concentration takes longer to decrease after they take the glucose drink than the non-diabetic's.
(2 marks)

- 3.3** If the diabetic person had exercised after taking the glucose drink would their blood glucose concentration have decreased more quickly or more slowly? Explain your answer. (1 mark)
- 3.4** Suggest how the blood glucose concentration of a Type I diabetic would differ from the Type II diabetic after having the glucose drink. (2 marks)
- 3.5** Suggest what time insulin is released in the non-diabetic. Explain your answer. (2 marks)
- 3.6** Blood glucose concentration continues to rise after the release of insulin. Why is this? (1 mark)
- 3.7** Describe how insulin initiates the uptake of glucose by binding to a target cell. (2 marks)
- 3.8** Describe how glycogenolysis is activated by hormones through the second messenger model. (5 marks)
- 4** The tubular fluid to blood plasma concentration ratio (TF/P ratio) is an index used to measure how well the kidney is working. If substances are able to pass freely from the glomerulus into the Bowman's capsule they will have a TF/P ratio of 1.0, as their concentration in the plasma is the same as in the initial tubular fluid.
- 4.1** Complete **Table 1** to show which of the following substances will have a TF/P ratio of 1.0 in a healthy kidney. The first two have been done for you.

Table 1

| Substance | TF/P ratio of 1.0 |
|---|--------------------------|
| <i>urea</i> | ✓ |
| <i>serum albumin (protein)</i> | X |
| <i>sodium ions (Na^+)</i> | |
| <i>glucose</i> | |
| <i>red blood cells</i> | |

- (2 marks)
- 4.2** The TF/P ratio of the protein serum albumin is normally less than 1.0 in a healthy kidney, meaning that the concentration of serum albumin is higher in the plasma than the tubular fluid. Explain why this is the case. (1 mark)
- 4.3** A patient has kidney failure as a result of high blood pressure. Her doctor prescribes diuretics to reduce her blood volume, which will reduce her blood pressure. Diuretics can reduce the amount of Na^+ that is reabsorbed from the nephron. Suggest how diuretics can be used to decrease blood volume. (4 marks)

Learning Objectives:

- Understand that there may be many alleles of a single gene.
- Know that genotype is the genetic constitution of an organism.
- Know that phenotype is the expression of the genetic constitution and its interaction with the environment.
- Know that alleles may be dominant, recessive or codominant.
- Know that in a diploid organism, alleles at a specific locus may be either homozygous or heterozygous.

Specification Reference 3.7.1

Tip: ‘Loci’ is the plural of ‘locus’.

1. Genetic Terms

This section is all about genes and how organisms pass them on to their offspring. But before you start exploring it, you really need to get to grips with the basic terms described below.

Basic terms and definitions

Genes and alleles

A **gene** is a sequence of bases on a DNA molecule that codes for a protein (polypeptide) which results in a characteristic.

You can have one or more versions of the same gene. These different versions are called **alleles**. There can be many different alleles of a single gene, but most plants and animals, including humans, only carry two alleles of each gene, one from each parent. The order of bases in each allele is slightly different — that’s because each allele codes for different versions of the same characteristic. Alleles are represented using letters.

Examples

- There are many different alleles for eye colour. The allele for brown eyes is shown using a B, and the allele for blue eyes uses b.
- Pea plants have a gene for seed shape. The allele for a round seed is shown using a R, and the allele for a wrinkled seed uses r.

Loci

Humans are **diploid organisms**. This means we have two copies of each chromosome — one from each parent. It’s why we have two alleles of each gene. The allele of each gene is found at a fixed position, called a **locus**, on each chromosome in a pair (see Figure 1).

Genotype

The genotype of an organism is its genetic constitution, or put another way, the different alleles an organism has. This could be a list of all its alleles but usually it’s just the alleles for one characteristic at a time.

Examples

- One person may have the genotype BB for eye colour and another person Bb.
- One pea plant might have the genotype RR for seed shape and another pea plant rr.

Phenotype

The phenotype of an organism is ‘the expression of the genetic constitution and its interaction with the environment’. This just means what characteristics an organism has as a result of both its genes and the effect the environment has on its genes.

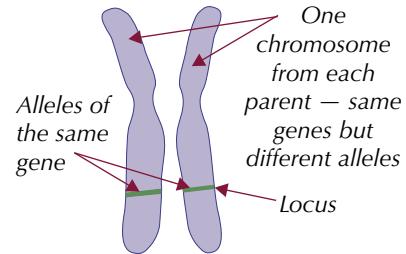


Figure 1: Diagram showing a locus on a pair of chromosomes.

Examples

- One person may have brown eyes and another may have blue eyes.
- One pea plant may have round seeds and another may have wrinkled seeds.

Exam Tip

You don't need to learn these examples for the exam — they're just here to help you understand the different terms.

Dominant and recessive alleles

A dominant allele is always expressed in the phenotype, even when there's only one copy of it. Dominant alleles are shown by a capital letter. Recessive alleles are those with characteristics that only appear in the phenotype if two copies are present. They're shown by lower case letters.

Examples

- The allele for brown eyes, B, is dominant, so if a person's genotype is Bb or BB they'll have brown eyes. The allele for blue eyes, b, is recessive, so a person will only have blue eyes if their genotype is bb.
- The allele for round seed shape, R, is dominant, so if a pea plant's genotype is Rr or RR it will have round seeds. The allele for wrinkled seed shape, r, is recessive, so a pea plant will only have wrinkled seeds if its genotype is rr.

Codominant alleles

Some alleles are both expressed in the phenotype because neither one is recessive. They are said to be codominant alleles.

Example

Horses can have alleles for white hair or coloured hair. Neither allele is recessive, so a horse with one copy of each allele will have a roan coat — a coat with a mixture of white hairs and coloured hairs.



Figure 2: A horse with a roan coat.

Homozygous and heterozygous

At each locus in a diploid organism, the genotype can be homozygous or heterozygous. If an organism carries two copies of the same allele, it's said to be homozygous at that locus. If an organism carries two different alleles for a gene, then it's heterozygous.

Examples

- The genotypes BB and bb are homozygous and the genotype Bb is heterozygous.
- The genotypes RR and rr are homozygous and the genotype Rr is heterozygous.

Tip: An organism can be homozygous at one locus and heterozygous at another.

Practice Questions — Application

Q1 In owl monkeys, the allele T codes for a tufted tail and t codes for a non-tufted tail. For each of the following genotypes, give the owl monkey's phenotype: A — Tt, B — TT, C — tt.

Q2 The yellow colour pea seed allele is dominant to the green allele.

- What would be the phenotype of a pea seed with the genotype Yy?
- Give the genotype of a homozygous pea seed that's yellow.
- Give the genotype of a green pea seed.

Exam Tip

Make sure you can answer these questions before you move on. If you don't understand these genetic terms now, you'll struggle with the rest of the section.

Learning Objective:

- Be able to use fully labelled genetic diagrams to interpret or predict the results of monohybrid crosses involving dominant, recessive and codominant alleles.

Specification Reference 3.7.1



Figure 1a: Photo of a fruit fly with normal wings.



Figure 1b: Photo of a fruit fly with vestigial wings.

Tip: The first set of offspring is called the F_1 generation.

Tip: A monohybrid cross with two homozygous parents will always produce all heterozygous offspring in the F_1 generation.

2. Genetic Diagrams — Simple Monohybrid Crosses

Genetic diagrams show how alleles could be passed on to the next generation.

What are genetic diagrams?

Diploid organisms have two alleles for each gene (see page 378).

Gametes (sex cells) contain only one allele for each gene — they're **haploid**.

When haploid gametes from two parents fuse together, the alleles they contain form the genotype of the diploid offspring that is produced.

Genetic diagrams can be used to predict the genotypes and phenotypes of the offspring produced if two parents are crossed (bred). You need to know how to use genetic diagrams to interpret or predict the results of various crosses, including monohybrid crosses.

Monohybrid inheritance

Monohybrid inheritance is the inheritance of a characteristic controlled by a single gene. **Monohybrid crosses** show the likelihood of the different alleles of that gene (and so different versions of the characteristic) being inherited by offspring of certain parents. The example below shows how wing length can be inherited in fruit flies.

Example

The allele for normal wings is dominant, so it's shown by a capital letter N. Any flies that have even one N allele will have normal wings.

The allele for vestigial (little) wings is recessive, so it's shown by the letter n. Only flies that have two n alleles will have vestigial wings.

The genetic diagram in Figure 2 shows a cross between one homozygous parent with normal wings (NN) and one homozygous parent with vestigial wings (nn). The normal winged parent can only produce gametes with the allele for normal wings (N). The vestigial winged parent can only produce gametes with the allele for vestigial wings (n).

Here's how to draw a genetic diagram for this cross:

Step 1: Make sure you're clear what the letters mean.

N — normal wings allele
n — vestigial (little) wings allele

Step 2: Show the parents' genotypes at the top.

Step 3: The middle circles show the possible gametes. Put one of each letter into a circle.

Step 4: The lines show all the possible ways the gametes could combine. Fill in the possible combinations in the bottom boxes.

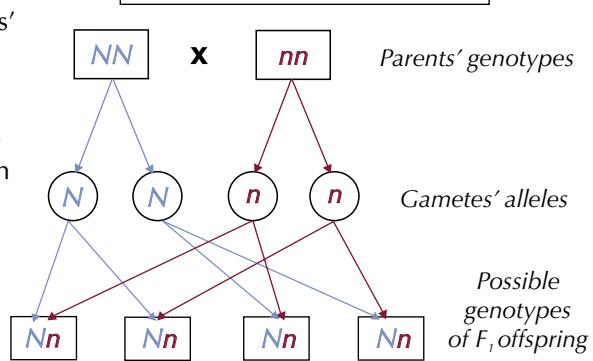


Figure 2: Genetic diagram showing a single generation monohybrid cross between homozygous parents.

All offspring produced are heterozygous (Nn), as one allele is inherited from each parent.

The genetic diagram in Figure 3 shows a cross between two parents from the F₁ generation (both heterozygous). Just follow the same steps as on the previous page, but this time the gametes produced by each F₁ offspring may contain the allele for either normal (N) or vestigial wings (n).

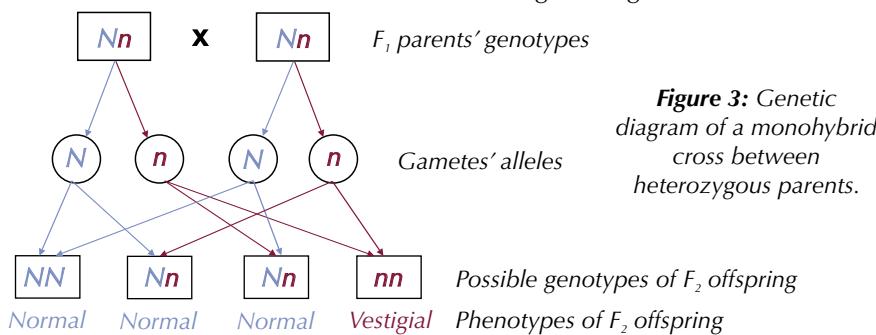


Figure 3: Genetic diagram of a monohybrid cross between heterozygous parents.

Exam Tip

If you draw a genetic diagram in the exam and you use letters that haven't been given to you in the question, you'll need to include a key to explain what those letters mean.

Phenotypic ratios

The phenotypic ratio is the ratio of different phenotypes in the offspring. Genetic diagrams allow you to predict the phenotypic ratios in F₁ and F₂ offspring.

Tip: The second set of offspring is called the F₂ generation.

Example — Maths Skills

Using the example above, there's a 75% chance the F₂ offspring will have the normal wings phenotype (genotype NN or Nn) and a 25% chance they'll have the vestigial wings phenotype (genotype nn). So you'd expect a 3 : 1 ratio of normal : vestigial wings in the offspring. This is the phenotypic ratio.

Usually whenever you do a monohybrid cross with two heterozygous parents you get a 3 : 1 ratio of dominant : recessive characteristics in the offspring. However, sometimes you won't get the expected (predicted) phenotypic ratio. For example, codominant alleles (see next page) and sex linkage (see p. 386) can both alter phenotypic ratios in the offspring of monohybrid crosses.

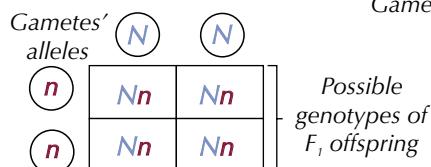
Tip: The 3 : 1 ratio is only an expected ratio. In practice, the phenotypic ratio in the offspring will probably be slightly different to this anyway, just by chance.

Punnett squares

A Punnett square is just another way of showing a genetic diagram. The Punnett squares below show the same crosses as p. 380 and above.

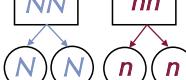
Example

Step 1: Work out the alleles the gametes would have.



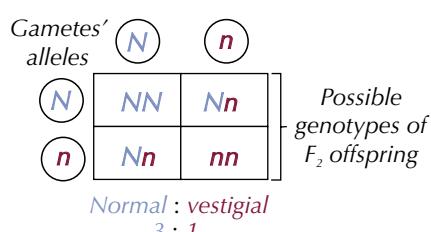
Parents' genotypes

NN nn



Gametes' alleles: N, n

Step 2: Cross the parents' gametes to show the possible genotypes of the F₁ generation — all heterozygous, Nn.



Ratio of phenotypes in F₂ offspring

Step 3: Cross the gametes of the F₁ generation to show the possible genotypes of the F₂ generation. The Punnett square shows a 75% chance that offspring will have normal wings and a 25% chance that they'll have vestigial wings, i.e. a 3 : 1 ratio.

Exam Tip

It's up to you whether you draw a diagram or a Punnett square in the exam, whichever you find easier. You must make sure you label your diagram though, so it's clear what you're trying to show.

Monohybrid inheritance of codominant alleles



Figure 4: A coloured scanning electron micrograph (SEM) of normal red blood cells (red) and sickle-shaped cells (pink).

Tip: When alleles show codominance they're represented in a slightly different way to normal — you show the main gene as a normal capital letter (H) and then the alleles as superscript capitals (H^S or H^N), because neither is recessive.

Exam Tip

If you're not given letters to use for a genetic diagram, just choose sensible ones yourself, e.g. T for tall dominant allele and t for dwarf recessive allele. Try to avoid using letters that look similar as capital and lower case letters, e.g. C and c.

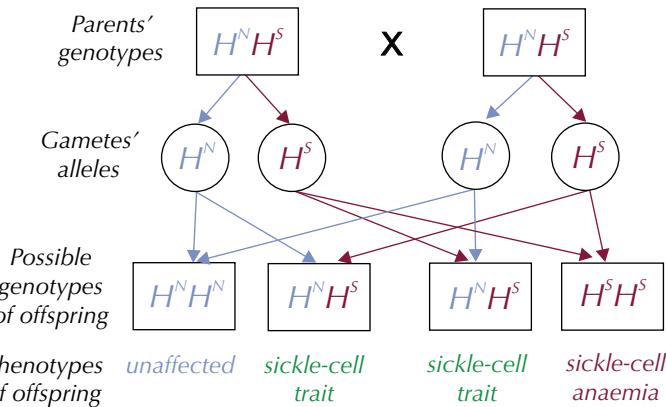
Exam Tip

If you're asked to find the probability of something, you can write it as a fraction (e.g. $\frac{3}{4}$), a decimal (e.g. 0.75) or a percentage (e.g. 75%).

Occasionally, alleles show codominance — both alleles are expressed in the phenotype, and neither one is recessive. One example in humans is the allele for sickle-cell anaemia, a genetic disorder caused by a mutation in the haemoglobin gene. It causes red blood cells to be sickle-shaped.

Example

People who are homozygous for normal haemoglobin ($H^N H^N$) don't have the disease. People who are homozygous for sickle haemoglobin ($H^S H^S$) have sickle-cell anaemia — all their blood cells are sickle shaped. People who are heterozygous ($H^N H^S$) have an in-between phenotype, called the sickle-cell trait — they have some normal haemoglobin and some sickle haemoglobin. The two alleles are codominant because they're both expressed in the phenotype. The genetic diagram in Figure 5 shows the possible offspring from crossing two parents with sickle-cell trait (heterozygous).



This cross has produced a 1 : 2 : 1 phenotypic ratio of 1 unaffected : 2 sickle-cell trait : 1 sickle-cell anaemia, or 1 unaffected homozygous : 2 heterozygous : 1 disorder homozygous.

Whenever you do a monohybrid cross with two heterozygous parents involving codominant alleles, you would expect to see a 1 : 2 : 1 ratio in the offspring.

Practice Questions — Application

- Q1 The allele for tall pea plants is dominant over the allele for dwarf pea plants. Give the possible genotype(s) of offspring produced if a homozygous tall pea plant is crossed with a homozygous dwarf pea plant. Show your working.
- Q2 Polydactyly is a genetic disorder where a baby is born with extra fingers or toes. The disorder is caused by a dominant allele. What is the probability of a baby being born with the condition if a person heterozygous for the disorder and a person without the disorder have a child? Show your working.
- Q3 In one organism, the alleles for skin colour show codominance. Any organisms that are homozygous with blue alleles are blue in colour. Organisms that are homozygous with yellow alleles are yellow in colour. Heterozygous organisms are yellow and blue striped. What colour ratio of organisms would be produced if a heterozygous parent was crossed with a homozygous blue parent? Show your working.

3. Genetic Diagrams — Multiple Allele and Dihybrid Crosses

Multiple allele crosses aren't much different to the monohybrid crosses you've already come across. They still only involve one gene, it's just the gene can have more than two alleles. If you want to, you can also use genetic diagrams to look at the inheritance of two genes simultaneously — this is called a dihybrid cross.

Multiple allele crosses

Inheritance is more complicated when there are more than two alleles of the same gene (multiple alleles).

Example

In the ABO blood group system in humans there are three alleles for blood type:

- I^O is the allele for blood group O.
- I^A is the allele for blood group A.
- I^B is the allele for blood group B.

Allele I^O is recessive. Alleles I^A and I^B are codominant — people with genotype $I^A I^B$ will have blood group AB.

Figure 1 shows a cross between a heterozygous person with blood group A and a heterozygous person with blood group B.

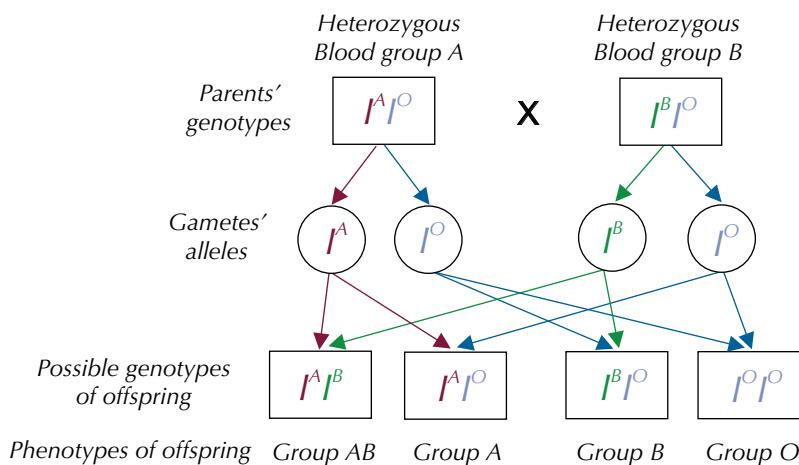


Figure 1: Genetic diagram showing the inheritance of blood group.

Any offspring could have one of four different blood groups (A, B, O or AB). So the expected phenotypic ratio is 1 : 1 : 1 : 1.

Learning Objectives:

- Be able to use fully labelled genetic diagrams to interpret or predict the results of crosses involving multiple alleles.
- Be able to use fully labelled genetic diagrams to interpret or predict the results of dihybrid crosses involving dominant, recessive and codominant alleles.

Specification Reference 3.7.1

Tip: Recessive blood groups are normally really rare, but it just so happens that loads of people in Britain are descended from people who were $I^O I^O$, so O's are really common.

Tip: Monohybrid crosses (see p. 380) look at the inheritance of one characteristic only.

Dihybrid crosses

Dihybrid inheritance is the inheritance of two characteristics, which are controlled by different genes. Each of the two genes will have different alleles.

Dihybrid crosses can be used to show the likelihood of offspring inheriting certain combinations of the two characteristics from particular parents.

The example on the next page is a dihybrid cross showing how seed shape and colour are inherited in pea plants.



Figure 2: Pea seeds can be wrinkled or round.

Tip: Each gamete should have one letter to represent each gene in the cross. So in a dihybrid cross, each gamete has two letters.

Tip: A dihybrid cross between a homozygous dominant parent and a homozygous recessive parent (e.g. RRYY \times rrry) will produce all heterozygous offspring in the F₁ generation.

Example

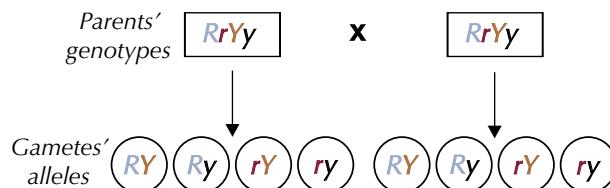
As you saw on page 378, the gene for seed shape has two alleles. The allele for round seeds (R) is dominant and the allele for wrinkled seeds (r) is recessive. The seed colour gene also has two alleles. The allele for a yellow seed (Y) is dominant and the allele for a green seed (y) is recessive.

The genetic diagram in Figure 3 shows a cross between two heterozygous parents — both have round and yellow seeds (RrYy).

Here's how to draw a genetic diagram for this cross:

Step 1: Make sure you're clear what the letters mean.

| | |
|---------------------------|-------------------------|
| <i>R</i> — round seeds | <i>Y</i> — yellow seeds |
| <i>r</i> — wrinkled seeds | <i>y</i> — green seeds |



Step 2: Work out the alleles the gametes would have.

Step 3: Cross the parents' gametes to show the possible offspring.

| RY | Ry | rY | ry | $Round\ and\ yellow\ seeds = RRYY, RrYY, RrYy, RRYy = 9$ |
|--------|--------|--------|--------|--|
| $RRYY$ | $RRYy$ | $RrYY$ | $RrYy$ | $Round\ and\ green\ seeds = RRYy, Rryy = 3$ |
| $RRYy$ | $RRyy$ | $RrYy$ | $Rryy$ | $Wrinkled\ and\ yellow\ seeds = rrYY, rrYy = 3$ |
| $RrYY$ | $RrYy$ | $rrYY$ | $rrYy$ | $Wrinkled\ and\ green\ seeds = rryy = 1$ |
| $RrYy$ | $Rryy$ | $rrYy$ | $rryy$ | $Phenotypic\ ratio: 9 : 3 : 3 : 1$ |

Figure 3: Genetic diagram showing a dihybrid cross between two heterozygous parents.

Usually, whenever you do a dihybrid cross with two heterozygous parents you get a 9 : 3 : 3 : 1 phenotypic ratio — that's 9 dominant both : 3 dominant first, recessive second : 3 recessive first, dominant second : 1 recessive both.

Dihybrid crosses and codominance

You can also do dihybrid crosses involving codominant alleles. They work in exactly the same way as the example above, but the phenotypic ratios produced are quite different (there are more than four possible phenotypes in the offspring).

Changes to phenotypic ratios

Even if neither of the genes involved in the dihybrid cross is codominant, you won't always get the expected 9 : 3 : 3 : 1 phenotypic ratio in the offspring of two heterozygous parents. This could be because of linkage or epistasis, both of which are covered on pages 386-392.

Practice Questions — Application

Q1 The colour of one species of moth is controlled by three alleles — pale typical (m), darkly mottled insularia (M') and nearly black melanic (M). The table below shows all possible genotype combinations and their phenotypic outcomes.

| Genotype | Phenotype |
|----------|------------------|
| mm | <i>Typical</i> |
| MM | <i>Melanic</i> |
| $M'M'$ | <i>Insularia</i> |
| mM | <i>Melanic</i> |
| mM' | <i>Insularia</i> |
| MM' | <i>Melanic</i> |

- Describe the dominance of the different alleles.
 - A homozygous melanic and a typical pale moth breed.
Show all the possible results of this cross.
- Q2 The striping pattern of cats can be determined by three alleles — Ta for Abyssinian, T for the mackerel phenotype and tb for blotched. Abyssinian is dominant to both of the other alleles, mackerel is dominant to blotched only and blotched is recessive to all.
(So the dominance of the alleles is Ta > T > tb.)
What are the possible striping patterns of offspring if a TaT cat and a tbtb cat breed together?
- Q3 In tomato plants, the allele for round fruit (F) is dominant to the allele for pear-shaped fruit (f). The allele for red fruit colour (R) is dominant to the allele for yellow fruit colour (r).
- Two tomato plants, heterozygous for fruit shape and colour, are crossed. Draw a Punnett square for this cross.
 - What is the expected ratio of round, red tomatoes to pear-shaped, yellow tomatoes?
- Q4 In cattle, the alleles for black colouring (B) and polled (no horns) (P) are dominant and the alleles for red colouring (b) and horns (p) are recessive.
- A black bull with no horns (BBPP) is crossed with a red cow with horns. What would the phenotypic ratio of the F_1 generation be?
 - Use a genetic diagram to show the expected phenotypic ratio in the offspring of a cross between two heterozygous black cattle with no horns.

Learning Objective:

- Be able to use fully labelled genetic diagrams to interpret or predict the results of crosses involving sex-linkage or autosomal linkage.

Specification Reference 3.7.1

Exam Tip

In the exam you could be asked to give the probability of producing a certain sex with a particular genotype, for example, a boy with blue eyes. You work out the probability of a child having blue eyes first and then divide it by 2, to include the 1 in 2 chance of having a boy. (This isn't the same thing as a sex-linked characteristic though.)

4. Linkage

There are two types of gene linkage, and they can both affect the phenotypic ratios of monohybrid and dihybrid crosses. You can use this variation from the expected ratios to identify that genes are linked.

Inheritance of sex-linked characteristics

The genetic information for biological sex is carried on two sex chromosomes. In mammals, females have two X chromosomes (XX) and males have one X chromosome and one Y chromosome (XY).

Figure 1 is a genetic diagram that shows how sex is inherited. From this you can see that the probability of having male offspring is 50% and the probability of having female offspring is 50%.

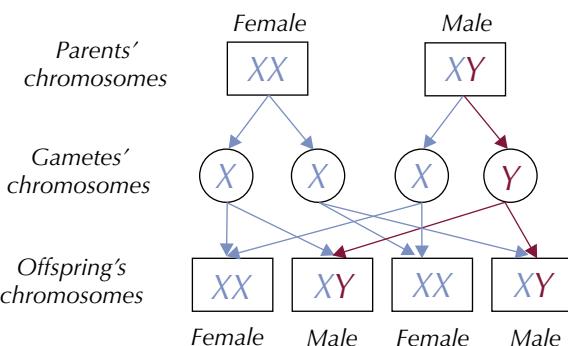


Figure 1: Genetic diagram showing the inheritance of sex.

Some characteristics are **sex-linked**. That means the alleles that code for them are located on a sex chromosome. The Y chromosome is smaller than the X chromosome and carries fewer genes. So most genes on the sex chromosomes are only carried on the X chromosome (called X-linked genes).

As males only have one X chromosome they often only have one allele for sex-linked genes. So because they only have one copy, they express the characteristic of this allele even if it's recessive. This makes males more likely than females to show recessive phenotypes for genes that are sex-linked.

Genetic disorders caused by faulty alleles located on sex chromosomes include colour blindness and haemophilia. The faulty alleles for both of these disorders are carried on the X chromosome and so are called X-linked disorders. Y-linked disorders do exist but are less common.

Example

Tip: The faulty allele for colour vision is represented by a lower case 'n', so you know it's a recessive allele.

Figure 2 on the next page shows a genetic diagram for colour blindness. Colour blindness is a sex-linked disorder caused by a faulty allele carried on the X chromosome. As it's sex-linked both the chromosome and the allele are represented in the genetic diagram, e.g. X^n , where X represents the X chromosome and n the faulty allele for colour vision. The Y chromosome doesn't have an allele for colour vision so is just represented by Y.

Females would need two copies of the recessive allele to be colour blind, while males only need one copy. This means colour blindness is much rarer in women than men. Females with one copy of the recessive allele are said to be **carriers**. A carrier is a person carrying an allele which is not expressed in the phenotype but that can be passed on to offspring.

Here's how to draw a Punnett square for the sex-linked cross between a carrier female and an unaffected male:

Step 1: Make sure you're clear what the letters mean. You need to show X and Y chromosomes too this time. You usually show them as a capital X and Y and then have the genes as superscript letters.

Step 2: Work out the alleles the gametes would have.

Step 3: Cross the parents' gametes to show the possible offspring.

Gametes' paternal alleles

N — normal colour vision allele
n — faulty colour vision allele
X — female **Y** — male

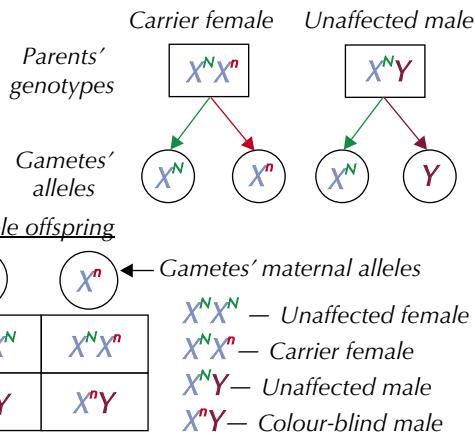


Figure 2: Punnett square showing the inheritance of colour-blindness.

In the example above, there's a 3 : 1 ratio of offspring without colour blindness : offspring with colour-blindness. But when a female carrier and a male without colour-blindness have children (as in this example), only their male offspring are at risk of being colour-blind. So you can also say that there's a predicted 2 : 1 : 1 ratio — of female offspring without colour-blindness : male offspring without colour-blindness : male offspring with colour-blindness. This ratio will change if a female carrier ($X^N X^n$) and a male with colour-blindness ($X^n Y$) have children. The predicted ratio will then be 1 : 1 — of offspring with colour-blindness : offspring without colour-blindness. The ratio will be the same for offspring of each sex. You only end up with this predicted ratio for a monohybrid F_2 cross with a sex-linked characteristic.

Linkage of autosomal genes

Autosome is the fancy name for any chromosome that isn't a sex chromosome. Autosomal genes are the genes located on the autosomes. Genes on the same autosome are said to be **linked** — that's because they'll stay together during the independent segregation of chromosomes in meiosis I, and their alleles will be passed on to the offspring together. The only reason this won't happen is if crossing over splits them up first. The closer together two genes are on the autosome, the more closely they are said to be linked. This is because crossing over is less likely to split them up.

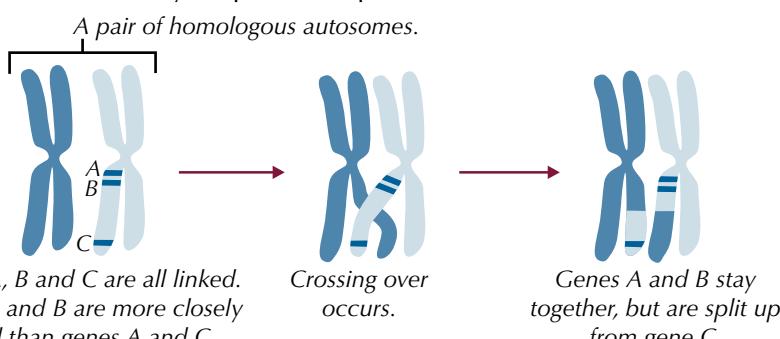
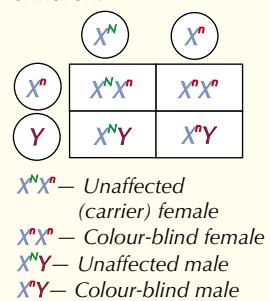


Figure 3a: Autosomal genes being split up during crossing over.

Tip: Males can't be carriers of X-linked disorders because they only have one copy of each chromosome, so if they have the allele they have the disease — whether it's recessive or not.

Tip: Here's a diagram to show the predicted phenotypic ratio of offspring if a female carrier and male with colour-blindness have children:

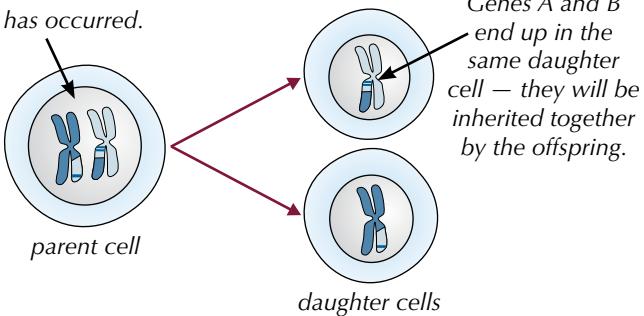


Two offspring are predicted to have colour-blindness and two aren't. This simplifies to a 1 : 1 ratio.

Tip: Independent segregation is the random division of homologous (paired) chromosomes into separate daughter cells during meiosis. Crossing over is when two homologous chromosomes 'swap bits'. It happens in meiosis I before independent segregation. You'll have learnt about both of these in Year 1 of your course.

The pair of autosomes after crossing over has occurred.

Figure 3b:
Independent segregation of autosomes during meiosis I.



If two genes are autosomally linked, you won't get the phenotypic ratio you expect in the offspring of a cross.

Tip: There's more about the expected phenotypic ratios for dihybrid and monohybrid crosses on pages 381 and 384.

Example

In a dihybrid cross between two heterozygous parents you'd expect a 9 : 3 : 3 : 1 ratio in the offspring. Instead, the phenotypic ratio is more likely to be that expected for a monohybrid cross between two heterozygous parents (3 : 1) because the two autosomally-linked alleles are inherited together. This means that a higher proportion of the offspring will have their parents' (heterozygous) genotype and phenotype.

So you can use the predicted phenotypic ratio to identify autosomal linkage.

Example

A scientist was investigating autosomal linkage between the genes for eye colour and wing length in fruit flies. The gene for normal wings (N) is dominant to the gene for vestigial wings (n) and the gene for red eyes (R) is dominant to the gene for purple eyes (r).

The first cross the scientist carried out was between flies homozygous dominant for both normal wings and red eyes (NNRR) and flies homozygous recessive for both vestigial wings and purple eyes (nnrr). The resulting offspring were all heterozygous for normal wings and red eyes (NnRr).

The second cross the scientist carried out was between these offspring (NnRr) and the flies homozygous recessive for vestigial wings and purple eyes (nnrr). He expected a 1 : 1 : 1 : 1 ratio as shown in Figure 4:

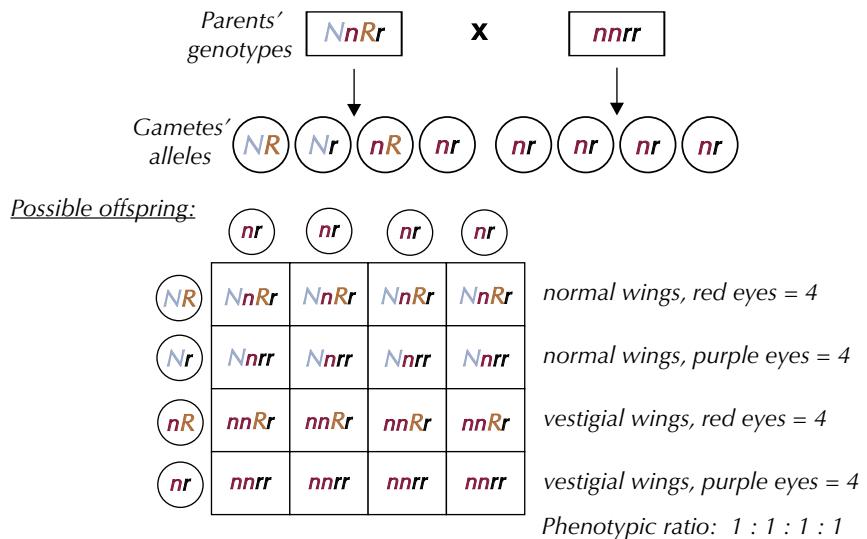


Figure 4: Genetic diagram showing the expected phenotypic ratio for a dihybrid cross between one heterozygous parent and one homozygous parent.

Tip: Crossing the offspring with one of the parents is known as a back cross.

Tip: Watch out — a 1 : 1 : 1 : 1 ratio is expected here because the cross is between a homozygous parent and a heterozygous parent not two heterozygous parents (which would be a 9 : 3 : 3 : 1 ratio).

However, the results the scientist got for the $\text{NnRr} \times \text{nnrr}$ cross showed an 8 : 1 : 1 : 8 ratio, as in the table:

| | Number of offspring |
|--|---------------------|
| Normal wings, red eyes (NnRr) | 1216 |
| Normal wings, purple eyes (Nnrr) | 152 |
| Vestigial wings, red eyes (nnRr) | 148 |
| Vestigial wings, purple eyes (nnrr) | 1184 |

$$\begin{aligned}\text{Phenotypic ratio} \\ = 8 : 1 : 1 : 8\end{aligned}$$

Tip: To give the ratio 1216 : 152 : 148 : 1184 in its simplest form, divide each number by the smallest number in the ratio (i.e. 148).

In order for the NnRr and nnrr genotypes to be so common in the offspring, the NR alleles and the nr alleles in the NnRr parent must have been linked. This means that the NnRr parent produced mostly NR and nr gametes. Some Nr and nR gametes were still made due to crossing over, but there were fewer Nnrr and nnRr offspring overall. As a result, a higher proportion of the offspring have their parents' phenotypes.

Exam Tip

In the exam you might get some genetic cross results that show linkage and have to explain them.

Practice Questions — Application

- Q1 Fragile X syndrome is an X-linked dominant disorder. A male and female, each with Fragile X syndrome, have a child. The female is heterozygous for the disorder. Give the possible genotypes and phenotypes of the child.
- Q2 Hypertrichosis pinnae (extremely hairy ears) was once thought to be a Y-linked characteristic. If this were true, why might a father with 'bald' ears whose child has hairy ears, be suspicious of his wife?
- Q3 In corn plants, the allele for glossy leaves (G) is dominant to the allele for normal leaves (g) and the allele for branching of ears (B) is dominant to the allele for no branching (b). A cross is carried out between a plant that is heterozygous for glossy leaves and branching of ears ($GgBb$) and a plant that is homozygous recessive for normal leaves and no branching (ggb).
- Use a genetic diagram to work out the expected phenotypic ratio in the offspring.
 - The results of the cross are shown in the table below.

| | Number of offspring |
|---|---------------------|
| Glossy leaves, lots of branching ($GgBb$) | 126 |
| Glossy leaves, no branching ($Ggbb$) | 81 |
| Normal leaves, lots of branching ($ggBb$) | 74 |
| Normal leaves, no branching (ggb) | 133 |

What is the observed phenotypic ratio in the offspring?

- Suggest why the observed ratio differs from the expected ratio.



Figure 5: A photo showing normal leaves and no branching in two ears of corn.

Practice Questions — Fact Recall

- What is the probability of having a female child?
- Some characteristics are sex-linked. What does this mean?
- Why are X-linked disorders more common in males than females?
- What is an autosome?
- Why are genes on the same autosome said to be linked?

Learning Objective:

- Be able to use fully labelled genetic diagrams to interpret or predict the results of crosses involving epistasis.

Specification Reference 3.7.1



Figure 1: A man with a widow's peak (a V-shaped hair growth). If this man were bald, you wouldn't be able to tell whether he had a widow's peak or not.

Tip: Epistatic genes are usually at different loci (different positions on chromosomes).

Tip: Remember the F_1 generation is the first generation and the F_2 generation is the second generation.

5. Epistasis

Just like linkage, epistasis affects the phenotypic ratios of dihybrid crosses.

What is epistasis?

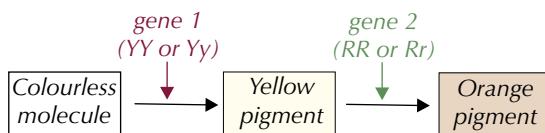
Many different genes can control the same characteristic — they interact to form the phenotype. This can be because the allele of one gene masks (blocks) the expression of the alleles of other genes — this is called **epistasis**.

Example 1 — Widow's peak

In humans a widow's peak (see Figure 1) is controlled by one gene and baldness by others. If you have the alleles that code for baldness, it doesn't matter whether you have the allele for a widow's peak or not, as you have no hair. The baldness genes are epistatic to the widow's peak gene, as the baldness genes mask the expression of the widow's peak gene.

Example 2 — Flower colour

Flower pigment in a plant is controlled by two genes. Gene 1 codes for a yellow pigment (Y is the dominant yellow allele) and gene 2 codes for an enzyme that turns the yellow pigment orange (R is the dominant orange allele). If you don't have the Y allele it won't matter if you have the R allele or not as the flower will be colourless. Gene 1 is epistatic to gene 2 as it can mask the expression of gene 2.



Phenotypic ratios for epistatic genes

Crosses involving epistatic genes don't result in the expected phenotypic ratios, e.g. if you cross two heterozygous orange flowered plants ($YyRr$) from the example above you wouldn't get the expected $9 : 3 : 3 : 1$ phenotypic ratio for a normal dihybrid cross.

The phenotypic ratio you would expect to get from a dihybrid cross involving an epistatic allele depends on whether the epistatic allele is recessive or dominant.

Recessive epistatic alleles

If the epistatic allele is recessive then two copies of it will mask (block) the expression of the other gene. If you cross a homozygous recessive parent with a homozygous dominant parent you will produce a $9 : 3 : 4$ phenotypic ratio of dominant both : dominant epistatic, recessive other : recessive epistatic in the F_2 generation.

Example

The flower colour example above is an example of a recessive epistatic allele. If a plant is homozygous recessive for the epistatic gene (yy) then it will be colourless, masking the expression of the orange gene. So if you cross homozygous parents you should get a $9 : 3 : 4$ ratio of orange : yellow : white in the F_2 generation. You can check the phenotypic ratio is right using a genetic diagram, like the one in Figure 2 (see next page).

Key:

Y — yellow pigment **R** — orange pigment
y — no yellow pigment **r** — no orange pigment

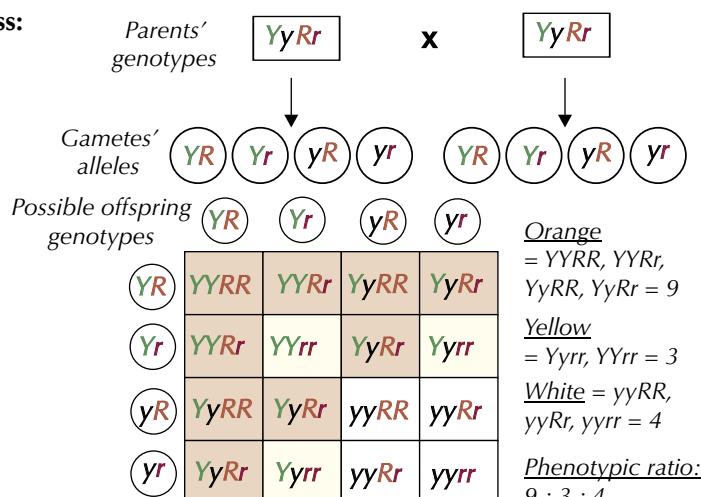
F₁ cross:**F₂ cross:**

Figure 2: Genetic diagram of a dihybrid cross with two heterozygous parents, involving a recessive epistatic gene.

Tip: All of the F₁ offspring have to have the genotype YyRr because the only gametes you can get from the parents are YR and yr.

Tip: You should be familiar with Punnett squares by now but if not, see page 381 for a recap.

Tip: This is a dihybrid cross because you're looking at the inheritance of two genes.

Dominant epistatic alleles

If the epistatic allele is dominant, then having at least one copy of it will mask (block) the expression of the other gene. Crossing a homozygous recessive parent with a homozygous dominant parent will produce a 12 : 3 : 1 phenotypic ratio of dominant epistatic : recessive epistatic, dominant other : recessive both in the F₂ generation.

Example

Squash colour is controlled by two genes — the colour epistatic gene (W/w) and the yellow gene (Y/y). The no-colour, white allele (W) is dominant over the coloured allele (w), so WW or Ww will be white and ww will be coloured. The yellow gene has the dominant yellow allele (Y) and the recessive green allele (y). So if the plant has at least one W, then the squash will be white, masking the expression of the yellow gene.

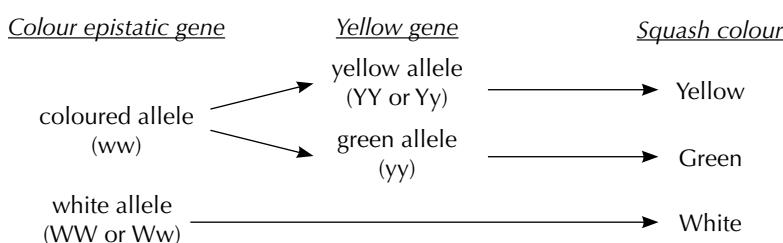


Figure 3: Diagram to show how squash colour is controlled by two genes.

So if you cross wwyy with WWYY, you'll get a 12 : 3 : 1 ratio of white : yellow : green in the F₂ generation. The genetic diagram to prove it is shown in Figure 5 (see next page).



Figure 4: These squash are yellow in colour so they must have the genotype wwYY or wwYy.

Key: W — white w — coloured Y — yellow y — green

F₁ cross: $WWYY \times wwyy \rightarrow$ All $WwYy$

F₂ cross: Parents' genotypes $WwYy \times WwYy$

Gametes' alleles $WY, Wy, wY, wy, WY, Wy, wY, wy$

Possible offspring genotypes

| | | | | |
|------|--------|--------|--------|--------|
| WY | $WWYY$ | $WWYy$ | $WwYY$ | $WwYy$ |
| Wy | $WWYy$ | $WWyy$ | $WwYy$ | Wwy |
| wY | $WwYY$ | $WwYy$ | $wwYY$ | $wwYy$ |
| wy | $WwYy$ | Wwy | $wwYy$ | $wwyy$ |

White = $WWYY, WWYy, WwYY, WwYy,$

$Wwy = 12$

Yellow = $wwYY, wwYy = 3$

Green = $wwyy = 1$

Phenotypic ratio:
 $12 : 3 : 1$

Exam Tip

If you set your crosses out like this in the exam, it'll help you keep track of what you're doing and it'll help the examiner follow your working out.

Figure 5: Genetic diagram of a dihybrid cross with two heterozygous parents, involving a dominant epistatic gene.



Figure 6: Chocolate and black coated Labrador retrievers.

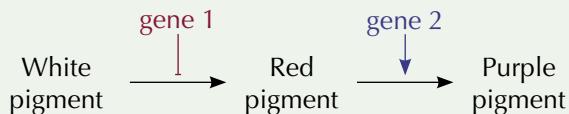
Practice Questions — Application

Q1 Coat colour in Labrador retrievers is controlled by two genes.

Gene 1 controls whether the dog can express dark pigment in its coat (E) or not (e). Gene 1 is epistatic over gene 2, which controls whether the dark pigment is black (B) or chocolate (b). Dogs that can't express dark pigment in their coat are yellow (golden) in colour.

- Write down all the possible genotypes for:
 - a black Labrador,
 - a chocolate Labrador,
 - a yellow Labrador.
- Describe and explain the phenotypic ratio produced in the F₂ generation if a black Labrador retriever (EEBB) breeds with a yellow Labrador retriever (eebb).

Q2 Petal colour in a species of flower is controlled by this pathway:



Gene 1 codes for a protein that prevents the formation of the red pigment. This means the dominant allele for gene 1 (W) causes the petals to be white and the recessive allele (w) causes red pigment to be made. Gene 2 codes for a protein that turns the red pigment into purple pigment. This means the dominant allele for gene 2 (P), causes the petals to be purple and the recessive allele (p) causes the petals to stay red. When a white flower (WWPP) is crossed with a red flower (wwpp), 48 white flowers, 12 purple flowers and 4 red flowers are produced in the F₂ generation.

- Is this an example of dominant or recessive epistasis?
- Explain the phenotypic ratio shown by the cross.
- Draw a genetic diagram to show this cross.

6. The Chi-Squared Test

The chi-squared test can be a bit tricky to get your head around, but take some time to work through these pages and you'll be fine.

What is the chi-squared test?

The chi-squared (χ^2) test is a statistical test that's used to see if the results of an experiment support a theory. First, the theory is used to predict a result — this is called the expected result. Then, the experiment is carried out and the actual result is recorded — this is called the observed result.

To see if the results support the theory you have to make a hypothesis called the **null hypothesis**. The null hypothesis is always that there's no significant difference between the observed and expected results. Your experimental result will usually be a bit different from what you expect, but you need to know if the difference is just due to chance, or because your theory is wrong. The χ^2 test is then carried out to compare the 'goodness of fit' of the observed and expected results (i.e. to compare how well the observed results match the expected results). The outcome either supports or rejects the null hypothesis.

Using the chi-squared test

You can use the χ^2 test in genetics to test theories about the inheritance of characteristics.

Example — inheritance of wing length experiment

Theory: Wing length in fruit flies is controlled by a single gene with two alleles (monohybrid inheritance). The dominant allele (N) gives normal wings, and the recessive allele (n) gives vestigial wings.

Expected results: With monohybrid inheritance, if you cross a homozygous dominant parent with a homozygous recessive parent, you'd expect a 3 : 1 phenotypic ratio of normal : vestigial wings in the F_2 generation.

Observed results: The experiment (of crossing a homozygous dominant parent with a homozygous recessive parent) is carried out on fruit flies and the number of offspring with normal and vestigial wings is counted.

Null hypothesis: There's no significant difference between the observed and expected results.

Chi-squared test: To find out if the results are significant, the **chi-squared value** is calculated (see below) and then compared to the **critical value** (see page 395). If the χ^2 test shows the observed and expected results are not significantly different, then we are unable to reject the null hypothesis — the data supports the theory that wing length is controlled by monohybrid inheritance.

Calculating the chi-squared value

Chi-squared (χ^2) is calculated using this formula:

$$\chi^2 = \sum \frac{(O - E)^2}{E}$$

O = observed result

E = expected result

Σ = the sum of...

The best way to understand the χ^2 test is to work through an example — there's one for testing the wing length of fruit flies, as explained above, on the next page.

Learning Objective:

- Be able to use the chi-squared (χ^2) test to compare the goodness of fit of observed phenotypic ratios with expected ratios.

Specification Reference 3.7.1

Exam Tip

In the exams, you could be asked to explain when you'd use a chi-squared test to analyse results. The test can be used whenever you have categorical data and you want to compare observed and expected results. See page 9 for more.



Figure 1: Karl Pearson — the English statistician who developed the chi-squared test.

Exam Tip

Don't worry, you won't be expected to calculate a χ^2 value in the written exams. You do need to be able to interpret the results of a chi-squared test though, so it's a good idea to understand how the test itself works.

Example — Maths Skills

Homozygous dominant flies (NN) are crossed with homozygous recessive flies (nn) and 160 offspring are produced in the F₂ generation.

- First the number of offspring expected (E) for each phenotype (out of a total of 160) is worked out using this equation:

$$E = \text{total no. of offspring} \div \text{ratio total} \times \text{predicted ratio}$$

A 3 : 1 phenotypic ratio of normal : vestigial wings is expected, so the ratio total is 3 + 1 = 4. Here are the expected results:

| Phenotype | Ratio | Expected result (E) |
|-----------------|-------|---------------------|
| Normal wings | 3 | 160 ÷ 4 × 3 = 120 |
| Vestigial wings | 1 | 160 ÷ 4 × 1 = 40 |

- Then the actual number of offspring observed with each phenotype (out of the 160 offspring) is recorded, e.g. 111 with normal wings:

| Phenotype | Ratio | Expected result (E) | Observed result (O) |
|-----------------|-------|---------------------|---------------------|
| Normal wings | 3 | 120 | 111 |
| Vestigial wings | 1 | 40 | 49 |

- The results are used to work out χ^2 . Taking it one step at a time:
 - $O - E$ is calculated for each phenotype (the expected result is subtracted from the observed result).

| Phenotype | Ratio | Expected result (E) | Observed result (O) | $O - E$ |
|-----------------|-------|---------------------|---------------------|----------------|
| Normal wings | 3 | 120 | 111 | 111 - 120 = -9 |
| Vestigial wings | 1 | 40 | 49 | 49 - 40 = 9 |

- Then the resulting numbers are squared:

| Phenotype | Ratio | Expected result (E) | Observed result (O) | $O - E$ | $(O - E)^2$ |
|-----------------|-------|---------------------|---------------------|---------|-------------|
| Normal wings | 3 | 120 | 111 | -9 | $-9^2 = 81$ |
| Vestigial wings | 1 | 40 | 49 | 9 | $9^2 = 81$ |

- These figures are divided by the expected results:

| Phenotype | Ratio | Expected result (E) | Observed result (O) | $O - E$ | $(O - E)^2$ | $\frac{(O - E)^2}{E}$ |
|-----------------|-------|---------------------|---------------------|---------|-------------|-----------------------|
| Normal wings | 3 | 120 | 111 | -9 | 81 | $81 \div 120 = 0.675$ |
| Vestigial wings | 1 | 40 | 49 | 9 | 81 | $81 \div 40 = 2.025$ |

- Finally, the numbers are added together to get χ^2 .

| Phenotype | Ratio | Expected result (E) | Observed result (O) | $O - E$ | $(O - E)^2$ | $\frac{(O - E)^2}{E}$ |
|--|-------|---------------------|---------------------|---------|-------------|-----------------------|
| Normal wings | 3 | 120 | 111 | -9 | 81 | 0.675 |
| Vestigial wings | 1 | 40 | 49 | 9 | 81 | 2.025 |
| $\sum \frac{(O - E)^2}{E} = 0.675 + 2.025 =$ | | | | | | 2.7 |

Tip: Don't forget — if you multiply a negative number by a negative number you get a positive number. So $-9^2 (-9 \times -9)$ is 81 and not -81.

The critical value

In your exam, you could be given a χ^2 value and be asked to determine whether there is a significant difference between the observed and expected results from an experiment. To do this you need to compare the χ^2 value to a critical value. The critical value is the value of χ^2 that corresponds to a 0.05 (5%) level of probability that the difference between the observed and expected results is due to chance.

Tip: There's more on levels of probability on page 9.

Finding the critical value

In the exam you might be given the critical value or asked to work it out from a table.

Example — Maths Skills

Figure 2 below is a chi-squared table — this shows a range of probabilities that correspond to different critical values for different **degrees of freedom** (explained below). Biologists normally use a **probability level** (P value) of 0.05 (5%), so you only need to look in that column.

| degrees of freedom | no. of classes | Critical values | | | | | |
|---|----------------|-----------------|------|------|------|-------|---|
| 1 | 2 | 0.46 | 1.64 | 2.71 | 3.84 | 6.64 | 10.83 |
| 2 | 3 | 1.39 | 3.22 | 4.61 | 5.99 | 9.21 | 13.82 |
| 3 | 4 | 2.37 | 4.64 | 6.25 | 7.82 | 11.34 | 16.27 |
| 4 | 5 | 3.36 | 5.99 | 7.78 | 9.49 | 13.28 | 18.47 |
| probability that result is due to chance only | | 0.50 | 0.20 | 0.10 | 0.05 | 0.01 | 0.001 (50%)(20%)(10%) (5%) (1%) (0.1%) |

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Figure 2: A chi-squared table.

In order to find the critical value for the wing length experiment:

- First, the degrees of freedom for the experiment are worked out — this is the number of classes (number of phenotypes) minus one. There were two phenotypes, so the degrees of freedom = 2 – 1 = 1.
- Next, the critical value corresponding to the degrees of freedom (1 in this case) and a probability level of 0.05 is found in the table. By following the arrows in Figure 2 you can see that the critical value is **3.84**.

Exam Tip

The table of critical values you get given in the exam might look a bit different to this, but don't panic. It'll still contain all the information you need to answer the question.

Tip: The two phenotypes for the wing length experiment are normal wings and vestigial wings.

Tip: Some other statistical tests also use degrees of freedom (e.g. Student's t-test). They're worked out differently for different tests.

Tip: In this kind of statistical test, you can never prove that the null hypothesis is true — you can only 'fail to reject it'. This just means that the evidence doesn't give you a reason to think the null hypothesis is wrong.

Comparing the χ^2 value to the critical value

If your χ^2 value is larger than (or equal to) the critical value, then there is a significant difference between the observed and expected results — i.e. something other than chance is causing the difference. This means the null hypothesis can be rejected. If your χ^2 value is smaller than the critical value, then there is no significant difference between the observed and expected results — the null hypothesis can't be rejected. This is summarised in Figure 3.

χ^2 value \geq critical value = reject the null hypothesis
 χ^2 value $<$ critical value = fail to reject the null hypothesis

Figure 3: Possible outcomes of a chi-squared test.

Example — Maths Skills

The chi-squared value of 2.7 is smaller than the critical value of 3.84. This means that there's no significant difference between the observed and expected results. We've failed to reject the null hypothesis — so the theory that wing length in fruit flies is controlled by monohybrid inheritance is supported.

Tip: If the χ^2 value had been bigger than 3.84 then something else must have been affecting wing length — like epistasis or sex linkage.

Practice Questions — Application

Q1 The critical value for a chi-squared test is 5.99. Explain whether or not the difference between the observed and expected results would be significant if the calculated chi-squared value was:

- a) 6.20, b) 4.85.

For the following questions, you may need to use the χ^2 table below:

| Degrees of freedom | Probability (p) | | | | | |
|--------------------|-----------------|------|------|------|-------|-------|
| | 0.50 | 0.20 | 0.10 | 0.05 | 0.01 | 0.001 |
| 1 | 0.46 | 1.64 | 2.71 | 3.84 | 6.64 | 10.83 |
| 2 | 1.39 | 3.22 | 4.61 | 5.99 | 9.21 | 13.82 |
| 3 | 2.37 | 4.64 | 6.25 | 7.82 | 11.34 | 16.27 |
| 4 | 3.36 | 5.99 | 7.78 | 9.49 | 13.28 | 18.47 |

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Tip: Remember, the degrees of freedom here are just the number of classes minus one ($n - 1$).

Q2 A student is looking at the inheritance of pea shape (round vs. wrinkled) and pea colour (green vs. yellow) in pea plants.

His theory is that this is a simple case of dihybrid inheritance with no linkage or epistasis involved. He predicts that if this is the case, when two heterozygous plants are crossed, there will be a 9 : 3 : 3 : 1 ratio in the offspring. To test his theory, the student carries out this cross and looks at the phenotypes of the 128 offspring produced. Some of his results are shown in the table below. His null hypothesis is that there is no significant difference between the observed and expected results.

- a) Copy and complete the table to calculate χ^2 for this experiment:

| Phenotype | Ratio | Expected result (E) | Observed result (O) | O – E | $(O - E)^2$ | $\frac{(O - E)^2}{E}$ |
|------------------|-------|---------------------|---------------------|-------|-------------|-----------------------|
| Round, green | 9 | | 74 | | | |
| Round, yellow | 3 | | 21 | | | |
| Wrinkled, green | 3 | | | | | |
| Wrinkled, yellow | 1 | | 7 | | | |
| | | | | | $(O - E)^2$ | |

$$\chi^2 = \sum \frac{(O - E)^2}{E} =$$

- b) Find the critical value for this experiment and explain whether the null hypothesis can be rejected or not.

Q3 A flower can have red, white or pink flowers. If this is an example of codominance and two heterozygous plants were crossed, you would expect a 1 : 2 : 1 ratio of red : pink : white flowers in the offspring. In order to test this, a null hypothesis was made and the cross was performed. Of the 160 offspring produced, 92 had pink flowers, 24 had red flowers and 44 had white flowers. From these figures, a chi-squared test result of $\chi^2 = 8.6$ was obtained.

a) What should the null hypothesis be for this test?

b) Is this cross likely to be an example of codominance? Explain your answer.

Section Summary

Make sure you know...

- That there can be one or more versions of the same gene and that these are called alleles.
- That genotype is what alleles an organism has and that phenotype is how these alleles show themselves.
- That alleles whose characteristic is always shown in the phenotype are called dominant, that those only shown in the phenotype if you have two copies are called recessive, and that codominant alleles both show in the phenotype.
- That if a diploid organism has two different alleles at the same locus it's heterozygous, but if it has two copies of the same allele at the same locus it's homozygous.
- How to use genetic diagrams showing monohybrid crosses involving dominant, recessive and codominant alleles to make predictions about offspring.
- That the phenotypic ratio is the ratio of phenotypes in the offspring.
- That the typical phenotypic ratio for a monohybrid cross between two heterozygous parents is 3 : 1 of dominant : recessive characteristic and the typical phenotypic ratio for a cross between two heterozygous parents involving codominant alleles is 1 : 2 : 1 of homozygous for one allele : heterozygous : homozygous for the other allele.
- How to use genetic diagrams showing crosses involving multiple alleles and dihybrid crosses to make predictions about offspring.
- That a typical phenotypic ratio for a dihybrid cross between two heterozygous parents is 9 : 3 : 3 : 1 (dominant both : dominant first, recessive second : recessive first, dominant second : recessive both).
- How to use genetic diagrams to show the inheritance of sex-linked characteristics (the alleles that code for them are located on sex chromosomes) and recognise that sex linkage alters expected phenotypic ratios in the offspring of crosses.
- How to use genetic diagrams to identify linked genes on autosomes and recognise that autosomal linkage alters expected phenotypic ratios in the offspring of crosses.
- That epistasis is when the allele of one gene masks the expression of the alleles of other genes.
- What recessive epistasis is and that when the epistatic allele is recessive, crossing a homozygous recessive parent with a homozygous dominant parent will produce a 9 : 3 : 4 phenotypic ratio of dominant both : dominant epistatic, recessive other : recessive epistatic in the F₂ generation.
- What dominant epistasis is and that when the epistatic allele is dominant, crossing a homozygous recessive parent with a homozygous dominant parent will produce a 12 : 3 : 1 phenotypic ratio of dominant epistatic : recessive epistatic, dominant other : recessive both in the F₂ generation.
- When a chi-squared test is used and what a null hypothesis is.
- How to find the critical value from a chi-squared table and how to use these values to determine whether the difference between observed and expected results is significant or not, and whether or not to reject the null hypothesis.

Exam-style Questions

- 1 In mice, the allele for wild-type speckled coat colour, agouti (A), is dominant to the allele for solid coloured fur (a).
- 1.1 Several pairs of heterozygous agouti mice are crossed, producing 256 offspring. Assuming this is a normal case of monohybrid inheritance, with no linkage involved, how many of the offspring would you expect to have the agouti coat colour? (1 mark)
- 1.2 The alleles for coat colour (A and a), are actually controlled by another gene (P). If a mouse is homozygous recessive for this gene, it is unable to produce any pigmentation and so will be albino. Give the possible genotype(s) that will produce the albino phenotype. (1 mark)
- 1.3 A student produces a genetic diagram to show the phenotypic ratio produced in the F_2 generation if a homozygous dominant mouse (PPAA) breeds with a homozygous recessive mouse (ppaa). His results are shown in **Figure 1** below.

Figure 1

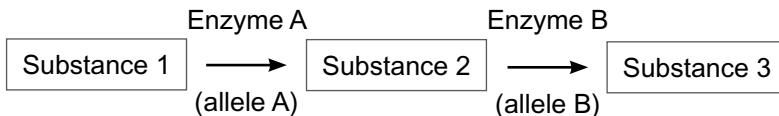
| | PA | pA | Pa | pa |
|----|------|------|------|------|
| PA | PPAA | PpAA | PPAa | PpAa |
| pA | PpAA | ppAA | PpAa | ppAa |
| Pa | PPAa | PpAa | PPaa | Ppaa |
| pa | PpAa | ppAa | Ppaa | ppaa |

The student concludes that this cross produces a phenotypic ratio of 9 : 3 : 3 : 1. This is incorrect. Give the phenotypic ratio that would be expected from this cross and explain why the student's conclusion is wrong.

(3 marks)

- 2 Yeast cells can convert substance 1 to substance 3 via the enzyme pathway shown in **Figure 2**. Two different gene loci control the pathway and each has two alleles. Having the dominant versions of alleles A and B means that the yeast cell will produce enzymes A and B as shown in **Figure 2**.

Figure 2



Yeast cells that lack either enzyme A or enzyme B cannot convert substance 1 to substance 3 and so cannot grow in media containing substance 1.

- 2.1** Complete the table by putting a tick (\checkmark) or a cross (\times) in the correct boxes below to show whether or not yeast cells with the following genotypes could grow on substance 1. The first one has been done for you

| Genotype | Growth on substance 1 |
|----------|-----------------------|
| AaBb | \checkmark |
| aaBb | |
| AAbb | |
| AABb | |

(1 mark)

- 2.2** Some of the cells that could not grow on substance 1 will grow if supplied with substance 2. Explain why, with reference to their genotype.

(3 marks)

- 3** Haemophilia is a sex-linked genetic disorder. It is caused by a faulty allele on the X-chromosome. The faulty allele (X^h) is recessive to the normal allele (X^H). A study was carried out into the inheritance of haemophilia. The phenotypes of children in families where the mother was a carrier of the disease (genotype $X^H X^h$) and the father was a haemophiliac (genotype $X^h Y$) were recorded.
- 3.1** Draw a genetic diagram to show why a 1 : 1 : 1 : 1 phenotypic ratio of haemophiliac male : haemophiliac female : carrier female : normal male was expected in the results of this study.

(3 marks)

Of the 272 children in this study, 130 were boys and 142 were girls. 61 of the boys and 70 of the girls had haemophilia. A chi-squared test was used to analyse the results. The results are shown in **Figure 3**. A table of critical values for chi-squared is shown in **Figure 4**.

Figure 3

| Phenotype | Ratio | Expected result (E) | Observed result (O) | $\frac{(O - E)^2}{E}$ |
|--------------------|-------|---------------------|---------------------|-----------------------|
| Carrier female | 1 | 68 | 72 | 0.24 |
| Haemophilic female | 1 | 68 | 70 | 0.06 |
| Normal male | 1 | 68 | 69 | 0.02 |
| Haemophilic male | 1 | 68 | 61 | 0.72 |
| Chi-squared = | | | | 1.04 |

Figure 4*

| Degrees of freedom | Probability (P) | | | | | |
|--------------------|-----------------|------|------|------|-------|-------|
| | 0.50 | 0.20 | 0.10 | 0.05 | 0.01 | 0.001 |
| 1 | 0.46 | 1.64 | 2.71 | 3.84 | 6.64 | 10.83 |
| 2 | 1.39 | 3.22 | 4.61 | 5.99 | 9.21 | 13.82 |
| 3 | 2.37 | 4.64 | 6.25 | 7.82 | 11.34 | 16.27 |

- 3.2** Use **Figure 3** and **Figure 4** to determine whether or not the difference between the observed and expected results is significant. Explain your answer.

(2 marks)

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Learning Objectives:

- Know that species exist as one or more populations.
- Know that a population is a group of organisms of the same species occupying a particular space at a particular time that can potentially interbreed.
- Understand the concepts of gene pool and allele frequency.
- Understand that the Hardy-Weinberg principle provides a mathematical model, which predicts that allele frequencies will not change from generation to generation.
- Know the conditions under which the Hardy-Weinberg principle applies.
- Be able to calculate allele, genotype and phenotype frequencies from appropriate data using the Hardy-Weinberg equation:
 $p^2 + 2pq + q^2 = 1$, where p is the frequency of one (usually the dominant) allele and q is the frequency of the other (usually recessive) allele of the gene.

Specification Reference 3.7.2

1. The Hardy-Weinberg Principle

A little bit of maths now... but I promise it's not too bad. Basically, you can use two fairly simple equations to work out allele, genotype and phenotype frequencies for a whole population — which is more useful than it sounds.

Gene pools and other terms

You need to get to grips with some key terms before you start playing around with equations and numbers:

- A **species** is defined as a group of similar organisms that can reproduce to give fertile offspring.
- A **population** is a group of organisms of the same species living in a particular area at a particular time — so they have the potential to interbreed. Species can exist as one or more populations, e.g. there are populations of the American black bear (*Ursus americanus*) in parts of America and in parts of Canada.
- The **gene pool** is the complete range of alleles present in a population. How often an allele occurs in a population is called the **allele frequency**. It's usually given as a percentage of the total population, e.g. 35%, or a decimal, e.g. 0.35.

What is the Hardy-Weinberg principle?

The Hardy-Weinberg principle is a mathematical model that predicts the frequencies of alleles in a population won't change from one generation to the next. But this prediction is only true under certain conditions:

- It has to be a large population where there's no immigration, emigration, mutations or natural selection (see page 405).
- There needs to be random mating — all possible genotypes can breed with all others.

The Hardy-Weinberg equations (see next page) are based on this principle. They can be used to estimate the frequency of particular alleles, genotypes and phenotypes within populations.

The Hardy-Weinberg equations can also be used to test whether or not the Hardy-Weinberg principle applies to particular alleles in particular populations, i.e. to test whether selection or any other factors are influencing allele frequencies. If frequencies do change between generations in a large population then there's an influence of some kind (see pages 403-404).

The Hardy-Weinberg equations

There are two Hardy-Weinberg equations you need to be able to use — one is used for working out allele frequency and the other one is usually used when you're dealing with genotype (and phenotype) frequencies. Both were designed to be used in situations where a gene has two alleles.

Allele frequency

The total frequency of all possible alleles for a characteristic in a certain population is 1.0 (100%). So the frequencies of the individual alleles (e.g. the dominant one and the recessive one) must add up to 1.

Here's that idea in an equation:

Where...

$$p + q = 1$$

p = the frequency of one allele (usually the dominant one)

q = the frequency of the other allele (usually the recessive one)

Tip: If the frequencies for two alleles add up to more than one, they're not alleles for the same gene (characteristic).

If they come to less than one, there are more than two alleles for that gene.

Genotype frequency

The total frequency of all possible genotypes for one characteristic in a certain population is 1.0. So the frequencies of the individual genotypes must add up to 1.0. But remember there are three genotypes — homozygous recessive, homozygous dominant and heterozygous. Here's the second equation:

Assuming p is dominant, and q is recessive, then:

$$p^2 + 2pq + q^2 = 1$$

p^2 = frequency of homozygous dominant genotype

$2pq$ = frequency of heterozygous genotype

q^2 = frequency of homozygous recessive genotype

Tip: Remember, homozygous dominant means two copies of the dominant allele (e.g. BB), homozygous recessive means two copies of the recessive allele (e.g. bb) and heterozygous means one copy of each allele (e.g. Bb).

These genotype frequencies can then be used to work out phenotype frequencies if you know how genotype relates to phenotype. Remember, genotype is the alleles an organism has (e.g. a plant could have the genotype Rr, where R codes for red flowers, and is dominant over r) and phenotype is the expression of this genotype in the environment (e.g. red flowers).

The Hardy-Weinberg equations also work if the two alleles are codominant (see page 379), or if you don't know which allele is recessive and which is dominant. In these situations, you can just make p represent one allele and q represent the other — it doesn't matter which is which, as long as you're consistent with how you use each letter, your calculations will work out fine.

Tip: There are two ways of making the genotype pq — you could get the p allele from the father and the q allele from the mother, or you could get the q allele from the father and the p allele from the mother. So the frequency of the heterozygous genotype is pq plus pq , which simplifies to $2pq$. If you get the p allele from both mum and dad you'll get the genotype $p \times p = p^2$. The same is true for the q allele.

Uses of the Hardy-Weinberg principle

The best way to understand how to use the principle and the equations is to follow through some examples.

Predicting allele frequency

You can figure out the frequency of one allele if you know the frequency of the other:

Example — Maths Skills

- A species of plant has either red or white flowers. Allele R (red) is dominant and allele r (white) is recessive. If the frequency of R is 0.4 in Population W, what is the frequency of r?

You know the frequency of one allele and just need to find the frequency of the other using $p + q = 1$ (where p = dominant allele, R, and q = recessive allele, r). So: $p + q = 1$

$$R + r = 1$$

$$0.4 + r = 1$$

$$r = 1 - 0.4 = 0.6$$

So the frequency of the r allele in Population W is **0.6**.

Exam Tip

Make sure you learn both equations and when to use them — you won't be given the equations in your exam.

Tip: It's a good idea to write down which letter represents which allele so you don't get confused halfway through your calculation.

Exam Tip

You may be given allele or genotype frequencies as percentages in the exam. To turn a percentage into a decimal just divide it by 100. For example, 90% as a decimal is $90 \div 100 = 0.9$.

Exam Tip

It's easier than it might seem to decide which equation to use. If you're given one allele frequency and asked to find the other it's the simple equation. If you know two out of the three genotype or phenotype frequencies, you can find the other frequency using the big equation. For anything else you'll probably need to use a combination of equations.

You can also figure out allele frequencies if you're given information about genotype (or phenotype) frequencies:

Example — Maths Skills

- There are two alleles for flower colour (R and r), so there are three possible genotypes — RR, Rr and rr. If the frequency of genotype RR is 0.56 in Population X, what is the allele frequency of r?
▪ You know that RR is the homozygous dominant genotype, so $RR = p^2$. You also know that the allele frequency for R = p , so: $p^2 = 0.56$
$$p = \sqrt{0.56} = 0.75, \text{ so } R = 0.75$$

You also know that $p + q = 1$, where p = the dominant allele, R, and q = the recessive allele, r. So: $p + q = 1$

$$R + r = 1$$

$$0.75 + r = 1$$

$$r = 1 - 0.75 = 0.25$$

So the frequency of the r allele (white) in Population X is **0.25**.

Predicting genotype frequency

Here you're after genotype, so it's p^2 , q^2 or $2pq$ you need to find:

Example — Maths Skills

- If there are two alleles for flower colour (R and r), there are three possible genotypes — RR, Rr and rr. In Population Y, the frequency of genotype RR is 0.34 and the frequency of genotype Rr is 0.27. Find the frequency of rr in Population Y.
▪ $p^2 + 2pq + q^2 = 1$, where p^2 = homozygous dominant genotype, RR, $2pq$ = heterozygous genotype, Rr, and q^2 = homozygous recessive genotype, rr. So: $p^2 + 2pq + q^2 = 1$
$$RR + Rr + rr = 1$$

$$0.34 + 0.27 + rr = 1$$

$$rr = 1 - 0.34 - 0.27 = 0.39$$

So the frequency of the rr genotype in Population Y is **0.39**.

Predicting phenotype frequency

You need to think about how phenotypes relate to genotypes here:

Example — Maths Skills

- If R is dominant and r is recessive, then a plant with a red flower phenotype could have the genotype RR or the genotype Rr. Plants with the genotype rr will have a white flower phenotype.
In population Z, the frequency of the genotype Rr is 0.23 and the frequency of the genotype rr is 0.42. Find the frequency of the red flower phenotype in population Z.
▪ The frequency of plants with red flowers in Population Z is equal to the genotype frequencies of RR and Rr added together.

The frequency of genotype $RR = p^2$, the frequency of genotype $Rr = 2pq$ and the frequency of the genotype $rr = q^2$. The only flowers with a white phenotype in the population have the genotype rr (as r is recessive), so the frequency of the phenotype red flowers is given by:

$$\begin{aligned} & p^2 + 2pq \\ & = RR + Rr \\ & RR + Rr + rr = 1 \\ & RR + Rr = 1 - rr \\ & RR + Rr = 1 - 0.42 = 0.58 \end{aligned}$$

So the frequency of red flowers in Population Z is **0.58**.

Tip: The more examples you practise, the more confident you'll be at working out allele, genotype and phenotype frequencies when it comes to your exam.

Predicting the percentage of a population that has a certain genotype

You're looking at genotype again, so it's ultimately something to do with p^2 , q^2 or $2pq$. But you might have to use a combination of equations to get there.

Example — Maths Skills

- The frequency of cystic fibrosis (genotype ff) in the UK is currently approximately 1 birth in 2500. Use this information to estimate the percentage of people in the UK that are cystic fibrosis carriers (Ff).
- To do this you need to find the frequency of the heterozygous genotype Ff , i.e. $2pq$, using both equations. (You can't just use the big one as you only know one of the three genotypes — q^2 .)

First calculate q :

Frequency of cystic fibrosis (homozygous recessive, ff) is 1 in 2500
 $ff = q^2 = \frac{1}{2500} = 0.0004$. So $q = \sqrt{0.0004} = 0.02$

Next calculate p :

Use $p + q = 1$, rearranged: $p = 1 - q = 1 - 0.02 = 0.98$

Then calculate $2pq$:

$2pq = 2 \times p \times q = 2 \times 0.98 \times 0.02 = 0.039$

The frequency of genotype Ff is 0.039, so the percentage of the UK population that are carriers is $0.039 \times 100 = 3.9\%$.

Showing if any external factors are affecting allele frequency

The Hardy-Weinberg principle predicts that the frequencies of alleles in a population won't change from one generation to the next as long as the population is large, there's no immigration, emigration, mutations or natural selection, and mating is totally random.

So if you use the Hardy-Weinberg equations to discover that allele frequency has changed from one generation to the next, then the Hardy-Weinberg principle doesn't apply to that population. This means that one (or more) of the factors listed above must be affecting allele frequency. For example, immigration might have occurred.

Tip: The effect of natural selection on allele frequency is covered in more depth on pages 405-407.

Example — Maths Skills

- If the frequency of cystic fibrosis is measured 50 years later it might be found to be 1 birth in 4500. Use this information to decide if the Hardy-Weinberg principle applies to this population.



Figure 1: G H Hardy (top) and Wilhelm Weinberg (bottom) actually came up with the ideas behind the Hardy-Weinberg principle independently from one another.

Tip: You don't always need to use all of the equation. You can just use the parts you want to find out, e.g. you know p and q and need to find the frequency of the heterozygous genotype, so just do $2pq$.

- Start by estimating the frequency of the recessive allele (f) in the population, i.e. q .

To calculate q :

Frequency of cystic fibrosis (homozygous recessive, ff) is 1 in 4500

$$ff = q^2 = \frac{1}{4500} = 0.00022$$

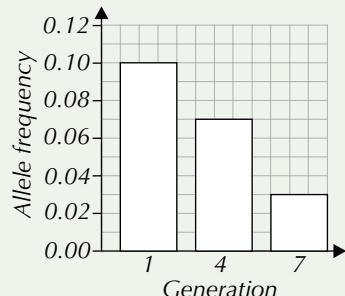
$$\text{So, } q = \sqrt{0.00022} = 0.01$$

The frequency of the recessive allele is now 0.01, compared to 0.02 currently (see previous page). As the frequency of the allele has changed between generations the Hardy-Weinberg principle doesn't apply.

Practice Questions — Application

- Q1 In a human population, the allele frequency for the recessive albino allele is measured over generations as shown in the bar chart below.

- Calculate the frequency of the pigmented (non-albino) allele in generation 1.
- Calculate the frequency of the heterozygous genotype in generation 1.
- Does the Hardy-Weinberg principle apply to this population? Explain your answer.



- Q2 Sickle cell anaemia is caused by a mutation to a single gene. People with sickle cell anaemia are homozygous for the sickle-cell allele, H^S . The sickle cell allele is codominant with the normal allele, H^N . Heterozygotes are said to have sickle cell trait. If the frequency of sickle cell anaemia in a population is approximately 1 birth in 500, what is the frequency of sickle cell trait?
- Q3 ADA deficiency is an inherited metabolic disorder caused by a recessive allele. The recessive allele frequency in a population is 0.16. What is the frequency of the homozygous dominant genotype in the same population?
- Q4 Seed texture in pea plants is controlled by two alleles, the dominant round allele and the recessive wrinkled allele. 31% of a population have wrinkled seeds. What percentage of the population have a heterozygous genotype?

Practice Questions — Fact Recall

- Define the term population.
- Explain what is meant by the term gene pool.
- Define the term allele frequency.
- Describe the Hardy-Weinberg principle and the conditions under which it is true.
- Write down the two Hardy-Weinberg equations and describe what each component represents.

2. Variation and Selection

The Hardy-Weinberg principle holds true if no external factors affect allele frequency. But that's not always the case in the real world...

Variation

Variation is the differences that exist between individuals. Variation within a species (also called 'intraspecific variation') means that individuals in a population can show a wide range of different phenotypes. Variation can be caused by genetic and/or environmental factors.

Although individuals of the same species have the same genes, they have different alleles (versions of genes) — this causes genetic variation within a species. The main source of this genetic variation is mutation, e.g. when changes in the DNA base sequence lead to the production of new alleles — see page 443. But genetic variation is also introduced during meiosis (through the crossing over of chromatids and the independent segregation of chromosomes) and because of the random fertilisation of gametes during sexual reproduction.

Variation within a species can also be caused by differences in the environment, like food, climate, or lifestyle. Most variation within a species is caused by a combination of genetic and environmental factors, but only genetic variation results in evolution.

Evolution

The frequency of an allele in a population changes over time — this is evolution. Evolution can occur by **genetic drift** (see page 410) or by **natural selection**.

Natural selection

Organisms face many pressures that affect their chances of surviving, such as predation, disease and competition. These are called **selection pressures**. Selection pressures create a struggle for survival. Because members of the same species have different alleles, there is variation between individuals, meaning that some are better adapted to the selection pressures than others. This means there are differential levels of survival and reproductive success in a population.

Individuals with a phenotype that increases their chance of survival are more likely to survive, reproduce and pass on their genes (including the beneficial alleles that determine their phenotype), than individuals with a different phenotype. This means that a greater proportion of the next generation inherit the beneficial alleles. They, in turn, are more likely to survive, reproduce and pass on their genes. So the frequency of the beneficial alleles in the gene pool increases from generation to generation.

Types of natural selection

The effect of natural selection on allele frequencies depends on the selection pressures acting on the population. There are three types of natural selection — **stabilising selection**, **directional selection** and **disruptive selection**.

Stabilising selection

This is where individuals with alleles for characteristics towards the middle of the range are more likely to survive and reproduce. It occurs when the environment isn't changing, and it reduces the range of possible phenotypes.

Learning Objectives:

- Know that individuals within a population of a species may show a wide range of variation in phenotype, and be able to explain why in terms of genetic and environmental factors.
- Know that the primary source of genetic variation is mutation, and that meiosis and the random fertilisation of gametes during sexual reproduction produce further genetic variation.
- Understand evolution as a change in allele frequencies within a population.
- Know that predation, disease and competition for the means of survival result in differential survival and reproduction, i.e. natural selection.
- Understand that those organisms with phenotypes providing selective advantages are likely to produce more offspring and pass on their favourable alleles to the next generation.
- Understand the effect of this differential reproductive success on allele frequencies within a gene pool.
- Know the effects of stabilising, directional and disruptive selection.

Specification Reference 3.7.3

Tip: You learnt about natural selection in Year 1 of your course.

Tip: How well-adapted an organism is to survive and reproduce successfully in its environment can be called its 'fitness' — so any alleles that enhance fitness are likely to increase in frequency via natural selection.

Tip: The breeding population is just the animals that are surviving, reproducing and passing on their alleles.

Exam Tip
Here the data shows phenotype, but in the exam you could get genotype or allele-specific data — the idea is still the same.

Tip: With data that shows stabilising selection, the mean stays in the middle. With data that shows directional selection, the mean moves in one direction or the other.

Exam Tip
Here the data is shown as a graph, but you could be given a table of data in your exam.

Example

In any mammal population there's a range of fur length. In a stable climate, having fur at the extremes of this range reduces the chances of surviving as it's harder to maintain the right body temperature, so mammals with very short or very long fur have a selective disadvantage. Mammals with alleles for average fur length are the most likely to survive, reproduce and pass on their alleles. These mammals have a selective advantage, so these alleles for average fur length increase in frequency.

Over time, the proportion of the population with average fur length increases and the range of fur lengths decreases — as shown in Figure 1. In the offspring graph the range of fur lengths has decreased, which results in a narrower graph. The proportion with average length fur has increased, resulting in a taller graph in the average fur length region.

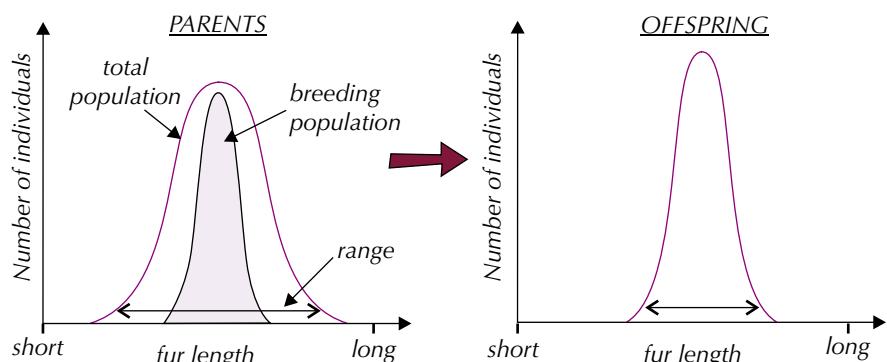


Figure 1: Graphs that show stabilising selection across generations.

Directional selection

This is where individuals with alleles for a single extreme phenotype are more likely to survive and reproduce. This could be in response to an environmental change.

Example

Cheetahs are the fastest animals on land. It's likely that this characteristic was developed through directional selection, as individuals that have alleles for increased speed are more likely to catch prey than slower individuals, meaning they're more likely to survive, reproduce and pass on their alleles.

Over time, the frequency of alleles for high speed increases and the population becomes faster — as shown in Figure 2. In the offspring graph, the average speed (dotted line) has moved towards the extreme, faster end.

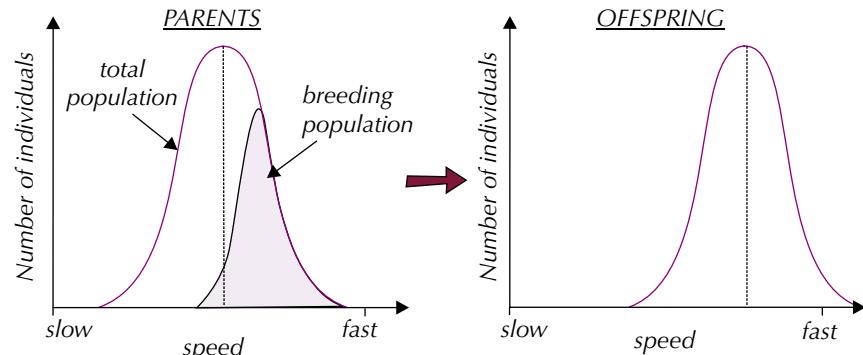


Figure 2: Graphs that show directional selection across generations.

Disruptive selection

This is where individuals with alleles for extreme phenotypes are more likely to survive and reproduce. It's the opposite of stabilising selection because characteristics towards the middle of the range are lost. It occurs when the environment favours more than one phenotype.

Example

In bird populations there's a range of beak sizes. Birds with large beaks are specialised to eat large seeds and birds with small beaks are specialised to eat small seeds. In an environment where the majority of seeds are large or small and very few (if any) are medium-sized, birds with medium-sized beaks may have a reduced chance of survival. This is because they are unable to eat either large or small seeds effectively. Birds with large or small beaks are more likely than birds with medium-sized beaks to survive, reproduce and pass on their alleles.

Over time, the alleles for a large beak and a small beak increase in frequency, but the alleles for a medium-sized beak decrease in frequency. The proportion of the population that have either small or large beaks increases — as shown in Figure 3.

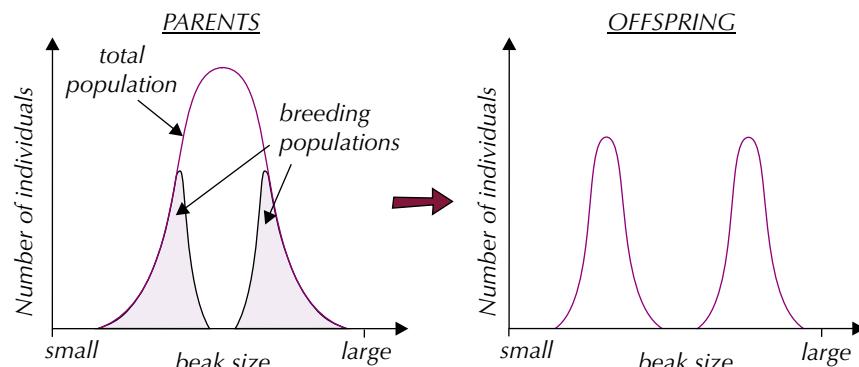


Figure 3: Graphs that show disruptive selection across generations.

Exam Tip

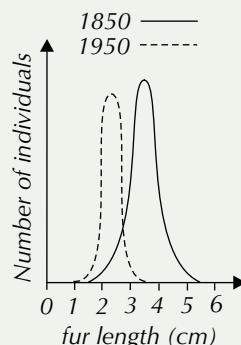
Make sure you know the differences between the three types of selection. You could be asked to interpret data that shows one of the types of selection and explain why the change has come about.

Exam Tip

If you're asked to explain any of the three types of selection, you need to make sure you get in the phrase 'more likely to survive, reproduce and pass on their alleles'.

Practice Question — Application

- Q1 The graph shows the fur length for a herd of caribou in 1850 and again 100 years later. In 1850 the population were moved from an area in the Arctic to an area much further south.
- Calculate the range of fur lengths for 1950.
 - What kind of selection does the graph show? Explain your answer.
 - Use your knowledge of selection to explain the results.



Tip: To help you answer Q1 c), remember the caribou were moved to an area further south than the cold Arctic.

Practice Questions — Fact Recall

- Give three ways in which genetic variation is caused.
- Explain how competition, predation and disease can alter allele frequencies within a gene pool over time.
- Explain the differences between stabilising and disruptive selection.

Learning Objectives:

- Know that reproductive separation of two populations can result in the accumulation of difference in their gene pools.
- Understand that when these genetic differences lead to an inability of members of the populations to interbreed and produce fertile offspring, new species arise from the existing species.
- Understand what is meant by allopatric and sympatric speciation.
- Be able to explain how natural selection and isolation may result in change in the allele and phenotype frequency and lead to the formation of a new species.
- Know that genetic drift causes changes in allele frequency in small populations and be able to explain why genetic drift is important only in small populations.
- Be able to explain how evolutionary change over a long period of time has resulted in a great diversity of species.

Specification Reference 3.7.3

Tip: A species is a group of individual organisms that can breed together to produce fertile offspring.

3. Speciation and Genetic Drift

Natural selection drives evolution, allowing species to change over time, and new species to evolve. For speciation to occur though, certain conditions need to be met. Populations can also change over time without selection acting at all, via the process of genetic drift.

What is speciation?

Speciation is the development of a new species from an existing species. It occurs when populations of the same species become **reproductively isolated** — changes in allele frequency cause changes in phenotype, which mean they can no longer interbreed to produce fertile offspring (see page 400).

Reproductive isolation can occur when a physical barrier, e.g. a flood or an earthquake, divides a population of a species, causing some individuals to become separated from the main population. This is known as geographical isolation. There is no gene flow (transfer of genes) between the two populations, which can lead to **allopatric speciation** (see below).

Alternatively, speciation can also occur when a population becomes reproductively isolated without any physical separation. This is known as **sympatric speciation** (see next page).

Allopatric speciation

Populations that are geographically separated will experience slightly different conditions. For example, there might be a different climate on each side of the physical barrier. The populations will experience different selection pressures and so different changes in allele frequencies could occur:

- Different alleles will be more advantageous in the different populations, so natural selection occurs. For example, if geographical separation places one population in a colder climate than before, longer fur length will be beneficial. Directional selection (see page 406) will then act on the alleles for fur length in this population, increasing the frequency of the allele for longer fur length.
- Allele frequencies will also change as mutations (see p. 443) will occur independently in each population.
- Genetic drift may also affect the allele frequencies in one or both populations (see page 410).

Over time, this can lead to speciation. The changes in allele frequency will lead to differences accumulating in the gene pools of the separated populations, causing changes in phenotype frequencies. Eventually, individuals from the different populations will have changed so much that they won't be able to breed with one another to produce fertile offspring — they'll have become reproductively isolated. The two groups will have become separate species (see Figure 1 on the next page).

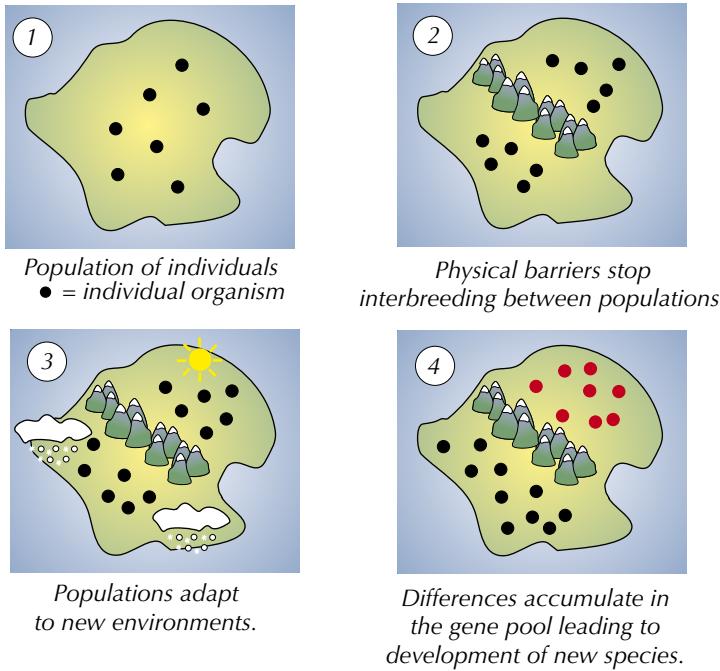


Figure 1: Diagram showing allopatric speciation.

Sympatric speciation

Sympatric speciation can occur when random mutations within a population prevent individuals that carry the mutation from breeding with other members of the population that don't carry the mutation. It doesn't involve geographical isolation.

It's generally thought that sympatric speciation is pretty rare, as it's difficult for a section of a population to become completely reproductively isolated from the rest of the population without being geographically isolated too (as is the case with allopatric speciation).

Example

Most eukaryotic organisms are diploid — they have two sets of homologous (matched) chromosomes in their cells. Sometimes, mutations can occur that increase the number of chromosomes. This is known as polyploidy. Individuals with different numbers of chromosomes can't reproduce sexually to give fertile offspring — so if a polyploid organism emerges in a diploid population, the polyploid organism will be reproductively isolated from the diploid organisms. If the polyploid organism then reproduces asexually, a new species could develop. Polyploidy can only lead to speciation if it doesn't prove fatal to the organism and more polyploid organisms can be produced. It's more common in plants than animals.



Figure 2: A drawing by Charles Darwin of four species of finch found in the Galapagos Islands. 'Darwin's finches' are often seen as a classical example of speciation.

Exam Tip

Make sure you're clear on the difference between sympatric and allopatric speciation, and can remember which is which. If it helps, you could think of Sympatric speciation happening in the Same place, and Allopatric speciation occurring in populations that are Away from each other.

Tip: Polyploidy can be a mechanism of reproductive isolation.

Mechanisms of reproductive isolation

Reproductive isolation occurs because changes in alleles, genotypes, and phenotypes prevent individuals with these changes from successfully breeding with individuals without them. These changes include:

Tip: Reproductive isolation is necessary for sympatric or allopatric speciation to take place.

Tip: Don't confuse geographical isolation with reproductive isolation. Populations that are geographically isolated are physically separated, but may still be able to reproduce if brought back together. Geographical isolation can lead to reproductive isolation if natural selection significantly changes the allele frequencies in the two separated populations.

Tip: All sorts of behavioural changes can cause reproductive isolation — e.g. different groups may develop different preferences about where they breed, which could prevent them from breeding with each other.

Exam Tip

Make sure you know the difference between genetic drift and natural selection. In natural selection, characteristics become more common if they increase an organism's likelihood of survival. In genetic drift, they become more common by chance.

Tip: Genetic drift tends to cause the genetic diversity of a population to decrease. Lack of genetic diversity may make species less able to adapt to future changes in their environment, so genetic drift can be a problem for small populations.

- Seasonal changes — individuals develop different flowering or mating seasons, or become sexually active at different times of the year. This means that they can't breed together, as they aren't reproductively active at the same time.
- Mechanical changes — changes in the size, shape or function of genitalia can prevent successful mating, preventing individuals from breeding.
- Behavioural changes — a group of individuals may, for example, develop courtship rituals that aren't attractive to the rest of the species, such as a change in song for birds. This prevents individuals from breeding with each other, even if they could do so successfully.

Evolution via genetic drift

Selection pressures can change the allele frequencies of a population over time. This is evolution by natural selection (see page 405).

Evolution also occurs due to genetic drift — this just means that instead of environmental factors affecting which individuals survive, breed and pass on their alleles, chance dictates which alleles are passed on. For this reason, genetic drift is sometimes called random drift.

Here's how it works:

- Individuals within a population show variation in their genotypes (e.g. A and B, see Figure 3).
- By chance, the allele for one genotype (B) is passed on to more offspring than the others. So the number of individuals with the allele increases.
- If by chance the same allele is passed on more often again and again, it can lead to evolution as the allele becomes more common in the population.

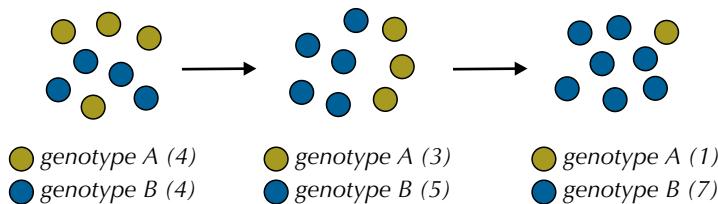


Figure 3: Diagram to show genetic drift in a population.

Genetic drift can lead to differences in allele frequency between two isolated populations. If enough differences in allele frequency build up over time, this could eventually lead to reproductive isolation and speciation.

Genetic drift and population size

Natural selection and genetic drift work alongside each other to drive evolution, but one process can drive evolution more than the other depending on the population size. Evolution by genetic drift usually has a greater effect in smaller populations where chance has a greater influence. In larger populations any chance factors tend to even out across the whole population.

Example — The evolution of human blood groups

Different Native American tribes show different blood group frequencies. For example, Blackfoot Indians are mainly group A, but Navajos are mainly group O.

Blood group doesn't affect survival or reproduction, so the differences aren't due to evolution by natural selection. In the past, human populations were much smaller and were often found in isolated groups. The blood group differences were due to evolution by genetic drift — by chance the allele for blood group O was passed on more often in the Navajo tribe, so over time this allele and blood group became more common.

Tip: The fact that genetic drift has affected these populations doesn't mean that speciation is taking place — blood group doesn't affect survival or cause reproductive isolation.

Speciation and diversity

The diversity of life on Earth today is the result of speciation and evolutionary change over millions of years.

To start with there was one population of organisms. The population was divided and the new populations evolved into separate species. The new species were then divided again and the new populations evolved into more separate species. This process has been repeated over a long period of time to create millions of new species (see Figure 4).

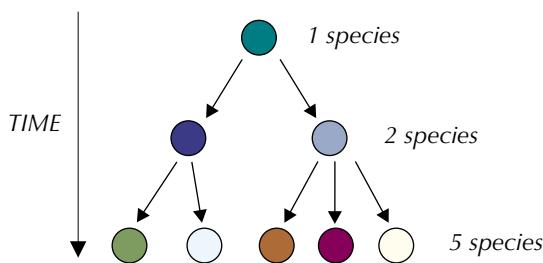


Figure 4: Over time, new species have evolved from existing ones to give the diversity of life we see on Earth today. This process can be shown in diagrams as an 'evolutionary tree'.



Figure 5: One of the earliest forms of life is thought to be a kind of bacteria that creates underwater towers of rock. These kinds of bacteria still exist today.

Practice Questions — Application

- Q1 Darwin observed 14 different species of finch on the Galapagos Islands in the Pacific Ocean. Each species of finch is unique to a single island and each island has a unique food source on it. Although the finches are all similar, the size and shapes of their beaks differ. Use your knowledge of selection and speciation to explain how these different species came about.
- Q2 The Mauritian pink pigeon is an endangered species. In the mid-1980s its numbers fell to less than 20, and a captive breeding programme was initiated. Why might the scientists running the programme have been concerned about the effects of genetic drift?

Tip: In a captive breeding program, organisms living in a safe environment, such as a zoo, are paired up and allowed to breed. The young may then be released back into the wild to increase the population size.

Q3 In the year 1990, a valley was flooded to create a reservoir. This also created an island, on which a small population of a rodent species was trapped. Unlike on the mainland, the rodent population on the island had no mammalian predators.

A group of scientists studied the island population in comparison to a larger population of the species on the mainland nearby. Every five years they collected data on the rodents' eye colour, size and behaviour.

- a) The rodent species has two eye colour phenotypes: black and pink, which are not thought to affect their probability of survival or chances of breeding. The scientists' observations of eye colour in the two populations are shown below:

| year | frequency of pink eyes in the population | |
|------|--|----------|
| | island | mainland |
| 1990 | 0.15 | 0.15 |
| 1995 | 0.08 | 0.16 |
| 2000 | 0.02 | 0.15 |
| 2005 | 0.00 | 0.14 |
| 2010 | 0.00 | 0.15 |

Tip: Take your time looking at any data you're given — make sure you really understand what the table in Q3 is showing you before you attempt to answer the questions.

Suggest an explanation for the observations shown in the table.

- b) The scientists found that by the end of 2010, the male rodents on the island were significantly larger than male rodents on the mainland. They observed that larger males were more likely to win in territorial fights with other males, and were more easily seen in the undergrowth.

Suggest why this change in male body size happened on the island but not on the mainland.

- c) Over the course of the study, the scientists also observed numerous behavioural changes in the rodents on the island. The scientists have suggested that these behavioural changes may have led to speciation. Describe how they could test whether speciation has occurred.

Practice Questions — Fact Recall

Q1 What is speciation?

Q2 Give two types of speciation and briefly describe each one.

Q3 Explain how behavioural changes may result in reproductive isolation.

Q4 Explain how evolution has led to the diversity of life on Earth.

Section Summary

Make sure you know...

- That a species is a group of organisms that can breed together to produce fertile offspring.
- That a population is a group of organisms of the same species occupying a particular space at a particular time that can potentially interbreed.
- That species exist as one or more populations and that the full range of alleles in a population is its gene pool.
- That how often an allele occurs in a population is called allele frequency, which can be given as a percentage or as a decimal.
- That the Hardy-Weinberg principle is a mathematical model that predicts that allele frequencies will not change from generation to generation, so long as it's a large population where there's no immigration, emigration, mutations or natural selection, and mating is totally random.
- The Hardy-Weinberg equations $p + q = 1$ and $p^2 + 2pq + q^2 = 1$, where p is the frequency of one (usually dominant) allele, q is the frequency of the other (usually recessive) allele, p^2 and q^2 are the frequencies of the two homozygous genotypes and $2pq$ is the frequency of the heterozygous genotype.
- How to use the Hardy-Weinberg equations and any data you are given to calculate allele, genotype and phenotype frequencies.
- That individuals within a population of a species show variation in phenotype due to genetic and environmental factors.
- That the main source of genetic variation is mutation, and that meiosis and the random fertilisation of gametes during sexual reproduction produce further genetic variation.
- That evolution is a change in allele frequencies in a population over time.
- That predation, disease, and competition create a struggle for survival, which results in differential survival and reproduction within populations due to natural selection — i.e. individuals with phenotypes that make them more likely to survive and reproduce are more likely to pass on their beneficial alleles to the next generation.
- That this differential reproductive success can cause a change in allele frequency within a gene pool (i.e. the frequency of beneficial alleles increases).
- That stabilising selection is where individuals in a population with alleles for characteristics towards the middle of the range are more likely to survive, reproduce and pass on their alleles.
- That directional selection is where individuals in a population with alleles for a phenotype at one extreme of the range are more likely to survive, reproduce and pass on their alleles.
- That disruptive selection is where individuals in a population with alleles for extreme phenotypes at either end of the range are more likely to survive, reproduce and pass on their alleles than individuals with alleles for phenotypes in the middle of the range.
- That speciation occurs when changes in allele frequencies in different populations of a species cause changes in phenotype that mean they can no longer interbreed to produce fertile offspring, and that this is called reproductive isolation.
- That allopatric speciation occurs when populations of the same species are geographically isolated and differences in the gene pools develop that can eventually lead to reproductive isolation.
- That sympatric speciation occurs when a random mutation causes reproductive isolation without geographic isolation.
- That genetic drift occurs when chance (rather than natural selection) leads to a change in allele frequencies in a population over time.
- That genetic drift is likely to have a greater affect in small populations rather than large populations because in large populations chance factors tend to even out across the whole population.
- That evolution and speciation over millions of years has led to the diversity of species on Earth today.

Exam-style Questions

- 1 Read the following passage:

Lake Apoyo in Nicaragua, Central America, is home to populations of many different species, including *Amphilophus citrinellus* and *Amphilophus zaliosus* — two species of fish. *A. zaliosus* is adapted to live in open water columns in the lake, whereas *A. citrinellus* lives on the lake bed. In each species, there is some variation in the phenotypes of individuals.

5

Lake Apoyo is an isolated lake, and is the only place in the world where *A. zaliosus* is found. It is thought that the *A. zaliosus* species evolved from the *A. citrinellus* population that originally inhabited the lake, and it has been suggested that disruptive selection may have contributed to the speciation. The two species are reproductively isolated and only tend to mate with fish of the same species.

10

Use the information above and your own scientific knowledge to answer these questions:

- 1.1 Define the term population (line 1).

(1 mark)

- 1.2 There is some variation in the phenotypes of individuals within both the *A. citrinellus* and the *A. zaliosus* species (lines 4-5). Other than the effect of environmental factors, explain how this variation in phenotypes could be caused.

(4 marks)

- 1.3 *A. zaliosus* and *A. citrinellus* are reproductively isolated (line 9).

Explain what this means and give **one** suggestion of how this may have occurred.

(3 marks)

- 1.4 It is thought that *A. zaliosus* evolved from *A. citrinellus* (line 7).

Name the type of speciation that is likely to have occurred. Explain your answer.

(2 marks)

- 1.5 It has been suggested that disruptive selection may have contributed to speciation in Lake Apoyo (lines 8-9). Suggest the reasoning behind this.

(5 marks)

- 2 The Amish population of North America descended from a small group of migrants. They live isolated from the surrounding population, and it is rare for people to migrate into the Amish community. The Amish population has an unusually high incidence of genetic disorders, including a rare form of dwarfism called Ellis van Creveld syndrome, which can lead to health problems and death in childhood.

- 2.1 Ellis van Creveld syndrome is caused by a recessive allele (e). In some Amish communities, the frequency of Ellis van Creveld syndrome may be as high as 5 births in every 1000. Use the Hardy-Weinberg equation to calculate the percentage of these communities that are **carriers** of Ellis van Creveld syndrome (genotype Ee). Show your working. Give your answer to **two decimal places**.

(2 marks)

- 2.2 The frequency of the Ellis van Creveld allele is much higher in some Amish communities than in the general population. What process is likely to have led to the high frequency of this allele? Give a reason for your answer.

(2 marks)

1. Ecosystems

All living things are found in places where they can cope with the local conditions, like the temperature and the availability of food. It's a fairly simple concept, but you need to be able to use some fancy words to describe it...

What is an ecosystem?

An **ecosystem** is all the organisms living in a **community** (see page 418), plus all the non-living (abiotic) conditions in the area in which they live. Ecosystems include both biotic and abiotic conditions:

- Biotic conditions are the living features of an ecosystem, for example, the presence of predators or food.
- Abiotic conditions are the non-living features of an ecosystem, such as the temperature and soil.

Example

In a freshwater ecosystem such as a lake, the biotic conditions would include the fish and the abiotic conditions would include the temperature of the water.

Ecosystems vary in size — they can be small, e.g. a pond, or large, e.g. an entire ocean. The place where an organism lives within an ecosystem is known as its **habitat** — for example, an area of reeds at the edge of a pond. Within a habitat each species has its own niche.

What is a niche?

A **niche** is the role of a species within its habitat, for example, what it eats, and where and when it feeds. The niche a species occupies includes:

- Its biotic interactions — e.g. the organisms it eats, and those it's eaten by.
- Its abiotic interactions — e.g. the temperature range an organism can live in, the time of day when an organism is active.

Every species has its own unique niche — a niche can only be occupied by one species. It may look like two species are filling the same niche (e.g. they're both eaten by the same species), but there'll be slight differences (e.g. variations in what they eat).

Example

Common pipistrelle bat

This bat lives throughout Britain on farmland, open woodland, hedgerows and urban areas. It feeds by flying and catching insects using echolocation (high-pitched sounds) at a frequency of around 45 kHz.

Soprano pipistrelle bat

This bat lives in Britain in woodland areas, close to lakes or rivers. It feeds by flying and catching insects using echolocation, at a frequency of 55 kHz.

It may look like both species are filling the same niche (e.g. they both eat insects), but there are slight differences (e.g. they use different frequencies for their echolocation).

Learning Objectives:

- Know that a community and the non-living components of its environment together form an ecosystem.
- Understand that ecosystems can range in size from the very small to the very large.
- Understand that, within a habitat, a species occupies a niche governed by adaptation to both biotic and abiotic conditions.

Specification Reference 3.7.4

Tip: You may know the expression "find your own niche", i.e. find the things you're good at. A species' niche is similar — it's the things a species does better than any other species.



Figure 1: The common (top) and soprano (bottom) bats are similar species but occupy different niches.

Tip: For more on competition take a look at pages 418-419.

Exam Tip

In questions where you're asked about the effect of natural selection on an adaptation, make sure you mention how survival or reproductive success of the species is affected.



Figure 2: The webbed paw of a North American river otter is an adaptation to abiotic conditions.

Tip: Metabolism is all the chemical reactions taking place inside an organism.



Figure 3: The use of twigs by chimpanzees to get termites out of termite holes is an adaptation to biotic conditions.

If two species try to occupy the same niche, they will compete with each other. One species will be more successful than the other, until only one of the species is left.

Adaptations

An **adaptation** is a feature that members of a species have that increases their chance of survival and reproduction. These features can be physiological (processes inside their body), behavioural (the way an organism acts) or anatomical (structural features of their body). For example, giraffes have long necks to help them reach vegetation that's high up. This increases their chance of survival when food is scarce. Organisms with better adaptations are more likely to survive, reproduce and pass on the advantageous alleles that determine these adaptations. This increases the frequency of these alleles in the population, which means the adaptations become more common. This is called **natural selection**.

Every species is adapted to use an ecosystem in a way that no other species can — it has its own unique niche. For example, only giant anteaters can break into ant nests and reach the ants. They have claws to rip open the nest, and a long, sticky tongue which can move rapidly in and out of its mouth to pick up the ants. Organisms are adapted to both the abiotic conditions (e.g. how much water is available) and the biotic conditions (e.g. what predators there are) in their ecosystem.

Examples

Adaptations to abiotic conditions

- Otters have webbed paws (see Figure 2) — this means they can both walk on land and swim effectively. This increases their chance of survival because they can live and hunt both on land and in water.
- Seals have a thick layer of blubber (fat) — this helps to keep them warm in the coldest seas. This increases their chance of survival because they can live in places where food is plentiful.
- Hedgehogs hibernate — they lower their rate of metabolism over winter. This increases their chance of survival because they can conserve energy during the coldest months.

Adaptations to biotic conditions

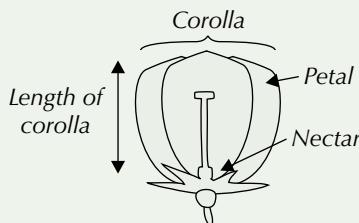
- Chimpanzees use twigs to fish termites out of termite mounds (see Figure 3). This increases their chance of survival because it gives them access to another source of food.
- Male frogs produce mating calls to attract females — this makes sure they attract a mate of the same species. This increases their chance of reproduction by making successful mating more likely.
- Some bacteria produce antibiotics — these kill other species of bacteria in the same area. This increases their chance of survival because there's less competition for resources.

Practice Questions — Application

Q1 The kangaroo rat is found in deserts. Its kidneys produce extremely concentrated urine.

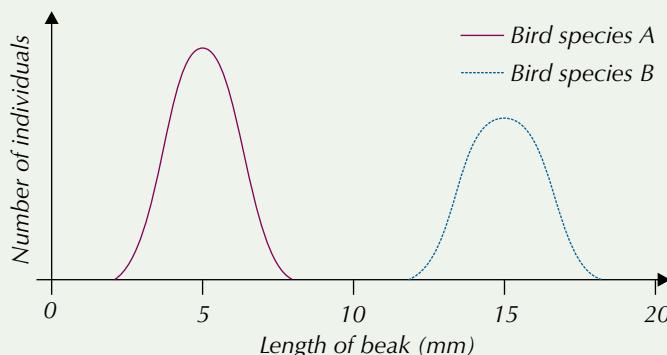
- a) Is the production of concentrated urine an adaptation to biotic or abiotic conditions?
- b) Suggest how this adaptation helps the kangaroo rat to survive.

- Q2** The length of probosci was studied in bees in a mountain habitat in Colorado. The bees were found to be dominated by three species: one with a long proboscis, one with a medium-sized proboscis and one with a short proboscis. The bees use their probosci to get nectar from the corolla of flowers. One such flower is shown below.



Tip: Probosci is the plural of proboscis — a long, straw-like sucking mouth part.

- Flowers with a variety of corolla lengths were observed on the mountain.
- Give one adaptation of the bees to a biotic condition in their habitat.
 - Suggest what would happen if another species of bee with a long proboscis was introduced to the mountain habitat.
- Q3** An investigation looked at the length of beaks in two closely related species of bird living in the same habitat. The birds eat seeds of similar plant species. The results are shown below.



- Describe the data shown by the graph.
- Suggest why the two bird species are able to share the same habitat.

Practice Questions — Fact Recall

- Q1** What is an ecosystem?
- Q2** What is a habitat?
- Q3** What is a niche?
- Q4** Give two examples of:
 - the biotic interactions of a species within its niche,
 - the abiotic interactions of a species within its niche.
- Q5** What is an adaptation?
- Q6** a) Describe the process in which adaptations become more common in a population.
b) What is the name given to this process?

Learning Objectives:

- Know that populations of different species form a community.
- Know that an ecosystem supports a certain size of population of a species, called the carrying capacity.
- Know that this population size can vary as a result of:
 - the effect of abiotic factors,
 - interactions between organisms: interspecific and intraspecific competition and predation.

Specification Reference 3.7.4

Exam Tip

Make sure you know what the terms 'population', 'community' and 'population size' mean.

Tip: Remember — abiotic factors are the non-living features of the ecosystem.

Tip: Remember — biotic factors are the living features of the ecosystem.

2. Variation in Population Size

Population sizes change all the time for lots of different reasons. To understand why this happens, first you need to know exactly what a population is...

Populations

A **population** is all the organisms of one species in a habitat.

Populations of different species in a habitat make up a **community**.

Example

All the foxes in a wood form a population. All of the species in the wood, like the foxes, squirrels, crab apple trees and so on, form a community.

Population size is the total number of organisms of one species in a habitat. This number changes over time because of the effect of various factors.

The maximum stable population size of a species that an ecosystem can support is called the **carrying capacity**. Carrying capacity varies as a result of both abiotic and biotic factors.

Abiotic factors and population size

The population size of any species varies due to abiotic factors, e.g. the amount of light, water or space available, or the temperature or the chemical composition of their surroundings. When abiotic conditions are ideal for a species, organisms can grow more quickly and reproduce successfully.

Example

When the temperature of a mammal's surroundings is the ideal temperature for metabolic reactions to take place, they don't have to use up as much energy maintaining their body temperature. This means more energy can be used for growth and reproduction, so their population size will increase.

When abiotic conditions aren't ideal for a species, organisms can't grow as fast or reproduce as successfully.

Example

When the temperature of a mammal's surroundings is significantly lower or higher than their optimum body temperature, they have to use a lot of energy to maintain the right body temperature. This means less energy will be available for growth and reproduction, so their population size decreases.

Biotic factors and population size

Population size can also vary because of biotic factors. These factors include interspecific competition, intraspecific competition and predation.

1. Interspecific competition

Interspecific competition is when organisms of different species compete with each other for the same resources. This can mean that the resources available to both populations are reduced, e.g. if they share the same source of food, there will be less available to both of them. This means both populations will be limited by a lower amount of food. They'll have less energy for growth and reproduction, so the population sizes will be lower for both species. If two species are competing but one is better adapted to its surroundings than the other, the less well adapted species is likely to be out-competed — it won't be able to exist alongside the better adapted species.

Example

Grey squirrels were introduced to the UK. They now compete with the native red squirrels for the same food sources and habitats. As they share the same source of food, there is less available to both of them. So in areas where both red and grey squirrels live, both populations are smaller than they would be if there was only one species there.

Since the introduction of the grey squirrel to the UK, the native red squirrel has disappeared from large areas. The grey squirrel has a better chance of survival because it's larger and can store more fat over winter. It can also eat a wider range of food than the red squirrel.

2. Intraspecific competition

Intraspecific competition is when organisms of the same species compete with each other for the same resources. It can cause a cyclical change in population size around the ecosystem's carrying capacity — where the population grows, shrinks, grows again and so on (see Figure 1). This is because the population of a species increases when resources are plentiful. As the population increases, there'll be more organisms competing for the same amount of space and food. Eventually, these resources become limiting. If the population grows beyond the carrying capacity, there won't be enough resources for all the organisms and the population will begin to decline. A smaller population then means that there's less competition for space and food, which is better for growth and reproduction — so the population starts to grow again. This cyclical pattern then continues...

Tip: Don't get inter- and intra-specific competition mixed up. If you're struggling, just remember — inter means different species, whereas intra means the same species.

Example

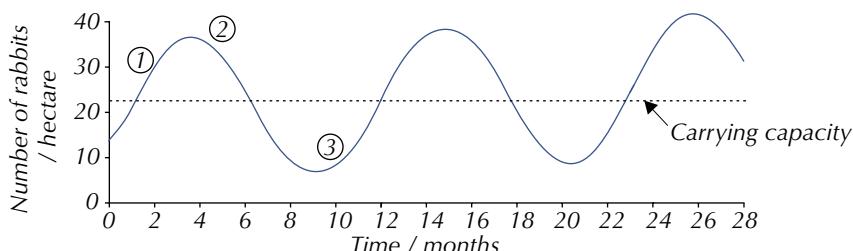


Figure 1: Intraspecific competition in a rabbit population.

1. There were lots of resources available so the population of rabbits grew.
2. The population grew so large that the resources became limiting — the carrying capacity of the ecosystem was exceeded. As there weren't enough resources, the rabbit population fell.
3. A smaller population of rabbits (below the carrying capacity) meant there was less competition, so the population of rabbits began to grow again.

Tip: Populations don't always overshoot their carrying capacities like this — a population can grow up to its carrying capacity and remain at a fairly stable size.

3. Predation

Predation is where an organism (the predator) kills and eats another organism (the prey), e.g. lions kill and eat (predate on) buffalo. The population sizes of predators and prey are interlinked — as the population of one changes, it causes the other population to change (see Figure 3 on the next page).

As the prey population increases, there's more food for predators, so the predator population grows. As the predator population increases, more prey is eaten so the prey population then begins to fall. This means there's less food for the predators, so their population decreases, and so on.



Figure 2: Predation of snowshoe hares by the lynx causes the populations of both species to fluctuate over time.

Example

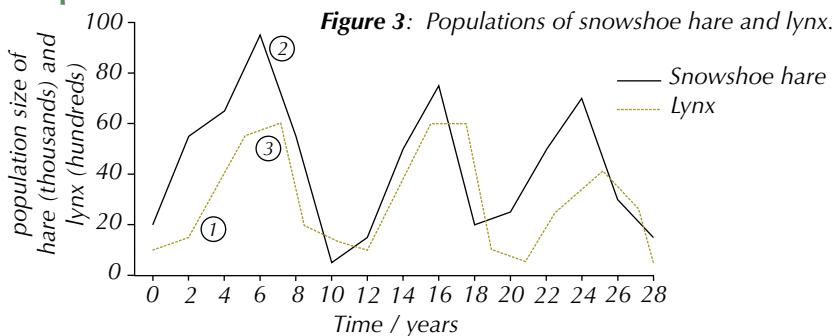


Figure 3: Populations of snowshoe hare and lynx.

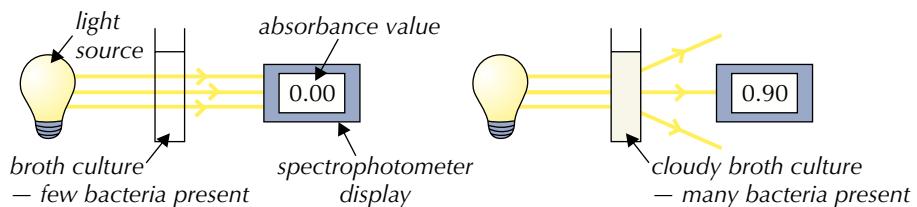
1. The lynx population grew after the snowshoe hare population increased. This is because there was more food available for the lynx.
2. Greater numbers of lynx ate lots of snowshoe hares, so the population of hares fell.
3. Reduced snowshoe hare numbers meant there was less food for the lynx, so the population of lynx fell.

Predator-prey relationships are usually more complicated than this though because there are other factors involved, like availability of food for the prey. E.g. it's thought that the population of snowshoe hare initially begins to decline because there's too many of them for the amount of food available. This is then accelerated by predation from the lynx.

Investigating population growth of bacteria

With enough food and space, the size of a population of microorganisms, e.g. bacteria, will grow at a steady rate. This can be investigated experimentally by growing bacteria in a liquid broth — a liquid containing the nutrients the bacteria need to grow. A liquid broth containing bacteria can be called a **broth culture**.

When light is passed through a sample of broth culture, some of it is scattered because bacteria are present — this reduces the amount of light passing through the culture. A machine called a spectrophotometer can measure the amount of light passing through a sample of the culture and produce an **absorbance value**. The more bacteria present in a culture, the less light will pass through to be detected by the spectrophotometer, producing a higher absorbance value (see Figure 4). So a broth culture sample with a high absorbance has a high number of bacteria present and vice versa.



Tip: A broth culture containing lots of bacteria will appear cloudy (turbid) — see Figure 4.

Tip: Spectrophotometers can also output a value for the percentage of light transmitted. E.g. a sample of clear liquid will have a very high percentage transmission because most of the light will pass through it.

The beginning of the experiment:
Most light passes straight through, so a low absorbance value is recorded.

A few hours later:
Less light passes through, so a high absorbance value is recorded.

Figure 4: Using a spectrophotometer to measure the absorbance of a broth culture.

If you plot a graph of absorbance against time, you'll get a graph with a shape like the one in Figure 5. This is called an **exponential graph** — it shows the bacteria doubling in number at regular intervals.

As the absorbance is proportional to the number of bacteria in a sample, you can convert the figures and draw a graph showing how the population of bacteria changes over time. However, the number of bacteria present will increase hugely over time, making it hard to draw a scale on your *y*-axis that can cover all the values you measure.

To get round this problem, you can take the **logarithm** of the number of bacteria at each point. These log values will be much smaller than the number of bacteria so they'll be easier to draw a scale for. If you plot the log of the number of bacteria against time, you'll get a straight line (Figure 6). You can use a graph like the one shown in Figure 6 to find the bacterial population at any given time on the *x*-axis.

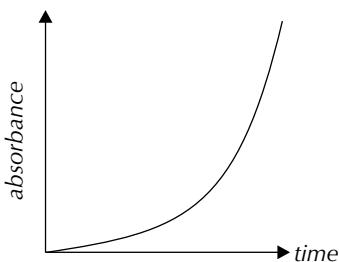


Figure 5: An exponential graph showing how absorbance increases with time.

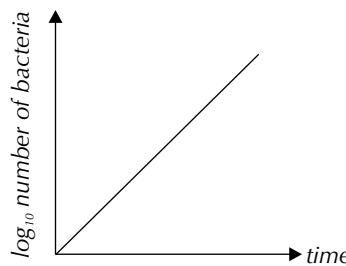


Figure 6: A graph showing how a population of bacteria changes over time using a log scale.

Tip: When bacteria are growing exponentially, you can work out how many bacteria are present in a population using the formula:
number of cells = initial number of cells $\times 2^n$, where '*n*' is the number of divisions.
E.g. if 300 bacteria grow exponentially for 7 divisions, you will end up with: $300 \times 2^7 = 38400$ cells
(or 3.84×10^4 cells).

Interpreting data values from a logarithmic scale

If you see microbial growth data plotted on a graph with the \log_{10} number of bacteria on the *y*-axis, it's possible to work out how many cells are present in the culture at a given time by finding the antilog (also known as the reverse or inverse log). To do this you need to use the 10^x button on your calculator. Simply press this, then enter the \log_{10} value at your chosen time. When you press equals, you'll get the number of cells.

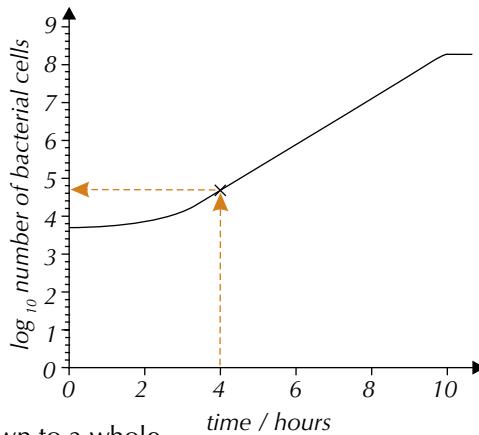
Example — Maths Skills

The graph shows part of a growth curve for a bacterial culture.

Estimate how many bacterial cells are present after 4 hours.

- You can see from the graph that at 4 hours, the \log_{10} number of bacterial cells is 4.7.
- To get the actual number of bacterial cells, you need to calculate the antilog using your calculator:
 $10^{4.7} = 50118$ cells present at 4 hours.

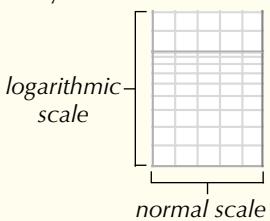
(You should round estimations down to a whole number, because you can't get parts of cells.)



Tip: Take a look back at p. 355 for more about log values. Remember, \log_{10} of 10 000 just means 'how many 10s need to be multiplied together to get 10 000?'. The answer is 4 because $10 \times 10 \times 10 \times 10 = 10 000$. There's a button for calculating log values on every scientific calculator. Spreadsheets can be used to calculate log values too.

Tip: 10^x is usually found written above the log button on a calculator (it's a second function of log). But different calculators work differently so make sure you know how to use yours.

Tip: Logarithmic graph paper has one axis with a logarithmic scale — the first 10 divisions on the paper gradually get closer together, until the 10th division when everything resets again. The pattern repeats every 10 divisions:



Tip: If you work out the number of cells present at two time points on a growth curve, you can use them to work out the rate of growth during that time period.

See pages 12-14 for more on working out rates from graphs.

Tip: In the exam, if you're given a graph with two y-axes like the one on the right, make sure you read the key carefully so you know which line relates to which axis.

Tip: With 'suggest' questions, like in Q2 on the right, you probably won't have learned the exact answer — you need to use the information you're given and apply your own knowledge to answer the question.

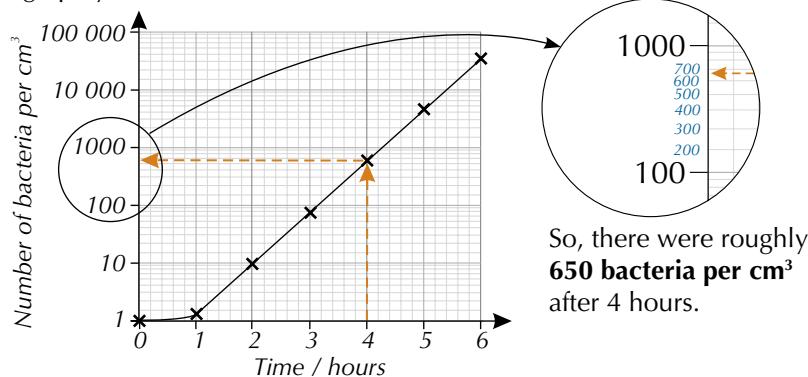
Logarithmic graph paper can also be used to plot microbial data on a logarithmic scale. The paper allows you to plot the actual number of bacteria rather than the \log_{10} values. This means you can read the number of bacteria present in the culture directly off the y-axis. Be careful though — the increments on the y-axis are not evenly spaced.

Example — Maths Skills

The graph below shows the growth of a bacterial culture over six hours.

Estimate how many bacteria per cm^3 were in the culture after four hours.

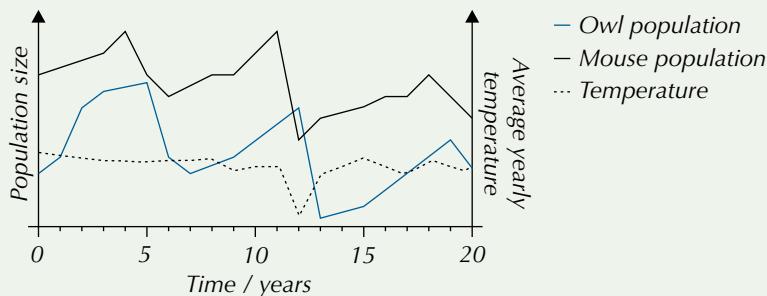
Draw a line up from 4 hours and across to the y-axis. The large square between 100 and 1000 is divided into nine sections, so the scale must be going up by 100 bacteria for each of those sections.



So, there were roughly **650 bacteria per cm^3** after 4 hours.

Practice Questions — Application

A team investigated changes in the size of a population of owls and a population of mice over twenty years. They also monitored changes in temperature. Their results are shown on the graph below.



- Q1 Give one factor affecting the population of owls which is biotic.
- Q2 Describe how the fall in temperature between years 11 and 12 may have affected the mouse population size, and suggest a reason for the change in population size.
- Q3 Explain how variation in the mouse population size over the twenty year period could have caused changes in the owl population size.

Practice Questions — Fact Recall

- Q1 What is: a) a population? b) a community?
- Q2 Define carrying capacity.
- Q3 Define interspecific competition and intraspecific competition.

3. Investigating Populations

There are lots of ways of investigating populations. Whichever method you use, you need to make sure your samples are random, you've carried out a risk assessment and you've thought about the ethics involved.

Abundance and distribution

Investigating populations of organisms involves looking at the abundance and distribution of species in a particular area.

Abundance

Abundance is the number of individuals of one species in a particular area (i.e. population size). Abundance can be estimated by simply counting the number of individuals in samples taken. There are other measures of abundance that can be used too:

- Frequency — the number of samples a species is recorded in, e.g. 70% of samples.
- Percentage cover (non-motile or slow-moving species only) — how much of the area you're investigating is covered by a species (see next page).

Distribution

Distribution is where a particular species is within the area you're investigating.

Random sampling

Most of the time it would be too time-consuming to measure the abundance (population size) and the distribution of each species present in the entire area you're investigating, so instead you take samples:

1. Choose an area to sample — a small area within the area being investigated.
2. Samples should be random to avoid bias. You can use a random number generator to ensure your samples are random (see below).
3. Use an appropriate technique to take a sample of the population (see pages 424-425).
4. Repeat the process, taking as many samples as possible. This will reduce the likelihood that your results are down to chance (see page 2).
5. The number of individuals for the whole area can then be estimated by taking the mean of the data collected in each sample and multiplying it by the size of the whole area. The percentage cover for the whole area can be estimated by taking the mean of all the samples.

Random number generators

If you were investigating populations in a field, you could pick random sample sites by dividing the field into a grid and using a random number generator and a random letter generator to select coordinates. This will give you coordinates at random, e.g. B7, E5, etc (see Figure 1). Then you just take your samples from these coordinates.

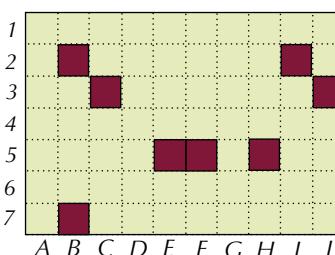


Figure 1: Randomly selected squares in a field.

Learning Objectives:

- Know that the size of a population can be estimated using:
 - randomly placed quadrats, or quadrats along a belt transect, for slow-moving or non-motile organisms,
 - the mark-release-recapture method for motile organisms and know the assumptions made when using the mark-release-recapture method.
- Be able to use given data to calculate the size of a population estimated using the mark-release-recapture method.
- Be able to investigate the effect of a named environmental factor on the distribution of a given species (Required Practical 12).

Specification Reference 3.7.4

Exam Tip

Make sure you know that the distribution of a species describes how individuals are spread out within an area.

Tip: Using tables of random numbers is another way of generating random numbers.

Tip: The point at which the running mean stabilises will be different in each area you study.

Running means

It's important that you take enough samples to give a good estimate. One way of doing this is to take a running mean — this is where you work out the mean of all the data each time you collect a new sample. Once the mean no longer changes by a large amount, you should have data that gives a realistic estimate for the whole area.

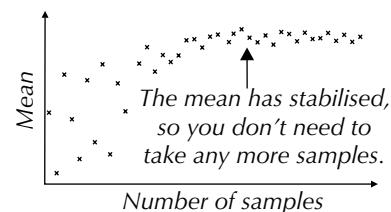


Figure 2: A running mean, plotted on a graph.

Tip: Non-motile organisms don't move around — they're fixed to a surface. Non-motile organisms can also be called sessile organisms. Motile organisms are able to move around freely, but they might move really slowly.

Tip: Putting your quadrat down where you happen to be standing, or even chucking it over your shoulder, doesn't count as taking a random sample. You're best off using a random number generator to select the coordinates to take your samples from (see previous page).



Figure 4: Quadrats can be used to measure the abundance of plant species in a field.

Methods for investigating populations

There are lots of different methods for studying populations of organisms, but you need to choose the most suitable one to use — this depends on the type of organism and its habitat. **Quadrats** and **transects** can be used for studying non-motile organisms, e.g. plants and corals, or slow-moving organisms like limpets. On the other hand, if you're studying more motile organisms, like insects, nets and traps are more appropriate.

Quadrats

A quadrat is a square frame, which is usually divided into a grid of 100 smaller squares by strings attached across the frame — see Figure 3.

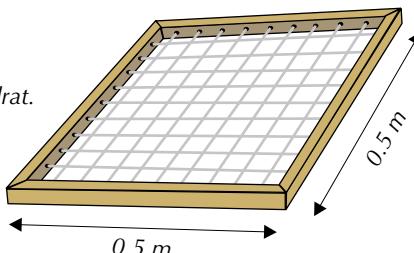


Figure 3: A 0.25 m^2 quadrat.

Quadrats are placed on the ground at different points within the area you're investigating. The species frequency (how often a species is found) or the number of individuals of each species is recorded in each quadrat.

The percentage cover of a species can also be measured by counting how much of the quadrat is covered by the species — you count a square if it's more than half-covered (see Figure 5). Percentage cover is a quick way to investigate populations and you don't have to count all the individual organisms.

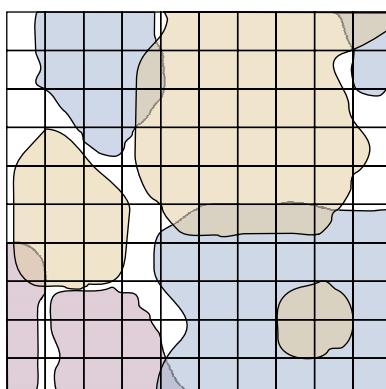


Figure 5: Measuring percentage cover using a quadrat.

- Species A: 42 squares = 42%
- Species B: 12 squares = 12%
- Species C: 47 squares = 47%

Quadrats are useful for quickly investigating areas with plant species that fit within a small quadrat — areas with larger plants and trees need very large quadrats.

Transects

You can use lines called transects to help find out how organisms are distributed across an area, e.g. how plant species change from a hedge towards the middle of a field. You need to know about:

1. **Belt transects** — quadrats are placed next to each other along the transect to work out species frequency and percentage cover along the transect.
2. **Interrupted belt transects** — instead of investigating the whole transect you can take measurements using a quadrat placed at regular intervals, e.g. every 2 metres. This can make it easier to cover a large distance.

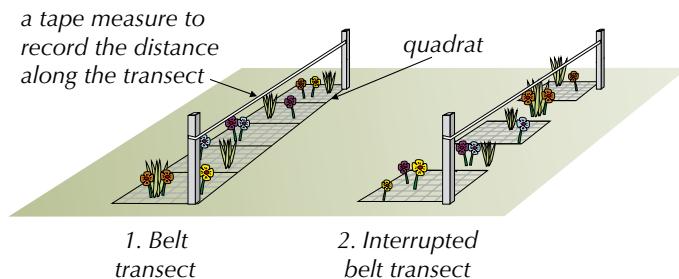


Figure 6: Diagram to show two different types of transect.

Tip: Interrupted belt transects are quicker to carry out than belt transects but give you less information. Which one it's best to do will depend on how much time you have and how long your transect is.

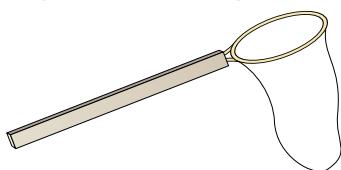
Tip: Transects can be used in any ecosystem, not just fields. For example, along a beach.

Capturing motile organisms

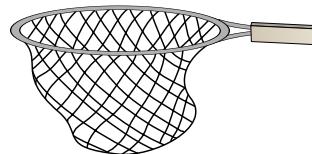
If you're investigating motile organisms, you might need to use equipment to capture them. The best method of capturing organisms will depend on what you're studying.

Examples

For flying insects, you'd use a sweep net (a net on a pole).

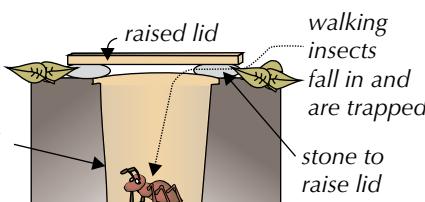


For aquatic animals you'd use a net.



For ground insects you'd use a pitfall trap (a steep-sided container that's sunk into the ground, see Figure 7).

flowerpot or similar container



Tip: When using equipment to take a sample of organisms in an area, it's important to use the same method each time. For example, with a sweep net you could sweep three times at shoulder height in each area you sample.



Practice Questions — Application

- Q1 A student is investigating the abundance of daisies in a field.
- She decides to use a quadrat to measure the percentage cover of daisies in the field. Describe how she could do this.
 - Describe how the student could take random samples using a quadrat.
- Q2 A scientist is investigating how the abundance of limpets on a rocky shore changes with distance from the sea. Describe a suitable method he could use to investigate this at low tide.

Figure 7: A pitfall trap with a cover to protect insects from rain and predators.

Mark-release-recapture

Mark-release-recapture is a method used to measure the abundance of more mobile species. Here's how it's done:

1. Capture a sample of a species using an appropriate technique (see previous page) and count them.
2. Mark them in a harmless way, e.g. by putting a spot of paint on them (see Figure 8) or by using an identification tag (see Figure 9).
3. Release them back into their habitat.
4. Wait a week, then take a second sample from the same population.
5. Count how many of the second sample are marked.
6. You can then use this equation to estimate the total population size:

$$\text{Total population size} = \frac{\text{Number caught in 1st sample} \times \text{Number caught in 2nd sample}}{\text{Number marked in 2nd sample}}$$



Figure 8: A turtle being marked with a spot of yellow paint before being released.

Exam Tip

Make sure you know the equation for estimating the total population size — don't rely on being given it in the exam.

Tip: The answer is rounded down to 38... so that you don't end up with a third of a woodlouse.

Example — Maths Skills

A pitfall trap was used to capture a sample of woodlice in a garden. The first sample contained 15 woodlice. The woodlice were marked and then released. A week later, a second sample of woodlice was collected from the same pitfall trap. There were 23 woodlice in the second sample, and 9 of them were marked.

$$\text{Total population size} = \frac{\text{Number caught in 1st sample} \times \text{Number caught in 2nd sample}}{\text{Number marked in 2nd sample}} = \frac{15 \times 23}{9} = 38.3$$

So the mark-release-recapture method gives an estimated total population size of 38 woodlice.



Figure 9: Birds that are caught can be marked by attaching a metal ring around one of their legs. These rings each have a code on them to identify individual birds. Capture may cause stress to some birds, which can raise ethical issues.

The accuracy of the mark-release-recapture method depends on a few assumptions:

- The marked sample has had enough time and opportunity to mix back in with the population.
- The marking hasn't affected the individuals' chances of survival (e.g. by making them more visible to predators), and the marking itself is still visible (e.g. it hasn't rubbed off).
- There are no changes in population size due to births, deaths and migration during the period of the study.

Some people think that capturing animals for study is unethical as it might cause them unnecessary stress. Also, if animals are put under too much stress during capture it could reduce their chances of survival after release, or influence them to avoid the trap in future. These would interfere with the accuracy of any estimates of population size made using the mark-release-recapture method. To minimise stress investigations should be planned so that organisms are treated carefully, and are kept and handled as little as possible.

Practice Question — Application

Q1 The mark-release-recapture method was used to estimate the size of a black beetle population in two different locations. On day one, the beetles were marked using white paint and then released. A second sample was captured the following day. The results are shown in the table below.

| Location | Size of first sample | Size of second sample | Number of marked beetles in second sample |
|----------|----------------------|-----------------------|---|
| A | 19 | 14 | 3 |
| B | 17 | 21 | 6 |

- Use the data in the table to estimate the total population size of beetles at:
 - location A,
 - location B.
- Are the estimates for these locations accurate? Explain your answer.

Tip: Remember, the method used to investigate the population of a particular organism depends on the organism itself. For example, using quadrats wouldn't be suitable for assessing bird populations.

Investigating the effect of an environmental factor on the distribution of a species

The distribution of species often changes within a particular area. For example, you might find more shade-loving plants at the edge of a field where they're sheltered by a tree, than in the centre where they're exposed to full sunlight. To find the effect of an environmental (abiotic) factor on the distribution of a species, you need to carry out a carefully planned investigation.

Example

You could investigate the effect of soil pH on marram grass in a coastal ecosystem. Here's how you could do it:

REQUIRED
PRACTICAL **12**

Method

- Place a tape measure in a straight line from the shore, heading inland. This will be your transect.
- Take a 1 m² quadrat divided into 100 squares (10 by 10).
- Starting from the shore, place the quadrat next to the tape measure. It doesn't matter where you position the quadrat relative to the tape measure, but you should do it the same way each time.
- Count the squares containing marram grass and record the result in a table as percentage cover (as shown on the next page). If you have time, take two repeat quadrat samples next to your initial quadrat and take a mean of your results. Alternatively, you could take a mean of the data from your whole class.
- At each sample point, you should also measure the pH (see next page) and record the results in the table.
- Repeat the observations every 10 m along the transect.



Figure 10: Marram grass is commonly found growing on sand dunes near the sea.

Tip: Always carry out a full risk assessment before doing any practical work. Have a look at the next page for some examples of safety issues that you need to think about before doing this Required Practical.

Tip: This is an example of an interrupted belt transect.

Tip: Being ‘in the field’ is when you’re outside in the area which you’re sampling. The term can be used for all sorts of locations, not just fields.

Tip: Label your samples carefully so you don’t get them mixed up.

Tip: You can use this sort of method to investigate how the distribution of any non-motile species changes as the environmental (abiotic) conditions change.

Measuring pH

If you have one, you can use a digital pH probe to take pH readings of sand or soil in the field. If you don’t have one, you can take a sample of sand/soil to test back at school. When you get back to school, you’ll need to sieve it to remove any debris, like twigs and leaves, and place it in a test tube. Add some barium sulfate, distilled water and pH indicator. Shake thoroughly and then leave to settle. Check the colour against a pH chart and record the result.

Results

Your results might look like this:

| Distance from shoreline / m | % cover | pH |
|-----------------------------|---------|-----|
| 0 | 0 | 8.5 |
| 10 | 11 | 8.4 |
| 20 | 27 | 8.0 |
| 30 | 40 | 7.6 |
| 40 | 58 | 7.5 |
| 50 | 55 | 7.5 |
| 60 | 21 | 7.1 |
| 70 | 15 | 7.0 |
| 80 | 8 | 6.8 |
| 90 | 7 | 6.6 |
| 100 | 0 | 6.5 |

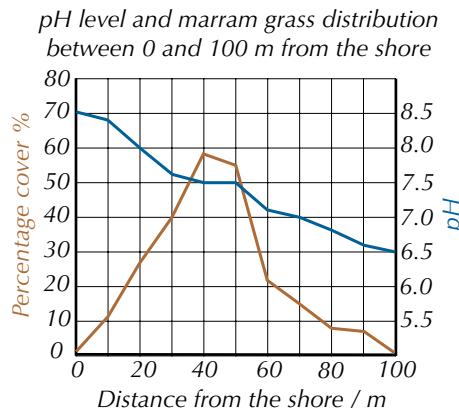


Figure 11: Example results showing how soil pH and abundance of marram grass might change with distance from the shore.

- pH (the blue line on the graph above) decreases as you move inland. This is because near the shore the sand/soil contains lots of shell fragments which are made of calcium carbonate, an alkaline compound. Further inland, the rotting vegetation adds organic matter to the soil, which is more acidic.
- At first, as pH decreases from 8.5 to 7.5, the percentage cover of marram grass (the orange line on the graph above) increases. After pH 7.5, marram grass percentage cover decreases as pH continues to decrease. You can’t say pH caused these trends in marram grass cover though — there could be other factors affecting it, including soil moisture content, salinity, and competition from other species.

Ethical issues

All fieldwork affects the environment where it’s carried out, e.g. lots of people walking around may cause soil erosion and marram grass can be killed by people trampling all over it. Investigations should be planned to have the smallest impact possible, e.g. people should restrict where they walk to the area being studied and try to avoid treading on the plants themselves.

Safety issues

When you’re carrying out fieldwork you expose yourself to risks. You need to think about what risks you’ll be exposed to, so you can plan ways to reduce the chance of them happening — this is called a risk assessment. For example, in this investigation you should use tide timetables, so you know what the local tide times are — low tide is the best time to work on a beach. Make sure you also wash your hands before eating, especially after handling soil.

Here are some examples of other fieldwork risks and ways to reduce them:

| | |
|------------------|---|
| Falls and slips | Wear suitable footwear for the terrain, e.g. wellies on wet or boggy ground and sturdy shoes on rough terrain to help stop you slipping. Make sure the study area isn't near any cliffs or on steep ground. |
| Weather | Check the weather forecast beforehand and take precautions, e.g. wear warm or waterproof clothing on cold or wet days and on hot days wear a sun hat and apply sun cream. If the weather is too severe, do the fieldwork another day. |
| Stings and bites | Wear insect repellent or, if you have an allergy, take medication with you. |



Figure 12: You can minimise the risk of slipping on boggy ground, e.g. by wearing appropriate footwear.

Practice Questions — Application

A scientist has been investigating the effect of salt spray from a road adjacent to an inland field. Her results are shown below.

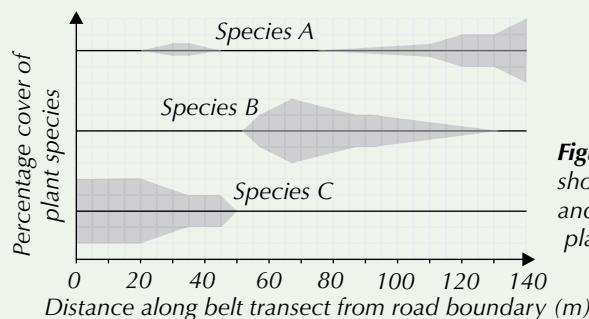


Figure 13: Kite diagram showing the distribution and abundance of three plant species in a field.

Tip: A kite diagram shows the distribution and abundance of organisms along a transect. The thickness of the kite shape shows the abundance — the thicker the kite shape, the more organisms there are.

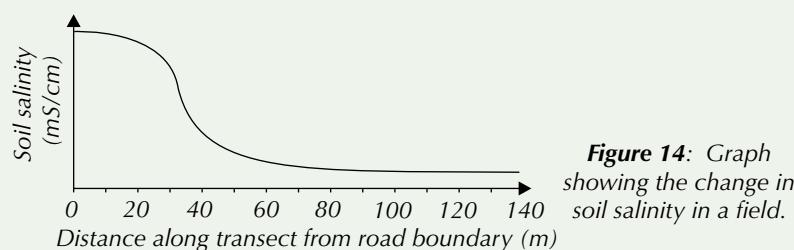


Figure 14: Graph showing the change in soil salinity in a field.

- Q1 Describe the data shown in the kite diagram and the graph.
- Q2 One of the plant species is normally found in coastal areas. Which species is this likely to be, A, B or C? Explain your answer.
- Q3 The scientist is unable to prove that salt spray from the road is responsible for the absence of species B between 0 and 20 m from the road using the data shown above. Explain why.

Practice Questions — Fact Recall

- Q1 What is meant by the terms: a) abundance, b) distribution?
- Q2 Name and describe two measures of abundance.
- Q3 What is: a) a quadrat, b) a belt transect?
- Q4 Give the equation for estimating total population size using data collected by the mark-release-recapture method.

Learning Objectives:

- Know that ecosystems are dynamic systems.
- Understand the process of primary succession, from colonisation by pioneer species to climax community.
- Understand that, at each stage in succession, certain species may be recognised which change the environment so that it becomes more suitable for other species with different adaptations.
- Understand that the new species which colonise an environment may change the environment in such a way that it becomes less suitable for the previous species.
- Understand that the changes that organisms produce in their abiotic environment can result in a less hostile environment and change biodiversity.

Specification Reference 3.7.4

Tip: Remember, biotic conditions are the living features of an ecosystem, e.g. the plant and animal communities. Abiotic conditions are the non-living features, such as light, CO_2 and water availability.

4. Succession

The plants and animals in an environment gradually change over long periods of time — and the environment itself changes too. This is due to a natural process called **succession**.

What is succession?

Ecosystems are **dynamic** — they're constantly changing. **Succession** is the process by which an ecosystem (see page 415) changes over time. Succession happens in a series of stages. At each stage, the plant and animal communities in an area slowly change the environmental conditions (for example, by making the soil more fertile), making the conditions more suitable for other species with different adaptations. This means that the **biotic conditions** change as the **abiotic conditions** change, causing one community of organisms to be succeeded (replaced) by another. There are two main types of succession — primary succession (see below) and secondary succession (see page 432).

Primary succession

Primary succession happens on land that's been newly formed or exposed, e.g. where a volcano has erupted to form a new rock surface, or where sea level has dropped, exposing a new area of land. There's no soil or organic material to start with, i.e. just bare rock.

Pioneer stage of succession

Primary succession starts when species colonise a new land surface. Seeds and spores are blown in by the wind and begin to grow. The first species to colonise the area are called **pioneer species**. The abiotic conditions are hostile (harsh) and only pioneer species can grow because they're specially adapted to cope with the harsh conditions.

Examples

Hostile abiotic conditions

- There is limited water available because there's no soil to retain water.
- There are few minerals or nutrients because there's no soil.
- There may be high light intensity, exposure to wind and rain, and fluctuating temperatures because the area is directly exposed to the Sun and the elements.

Pioneer species

- Marram grass (see p. 427) can grow on sand dunes near the sea because it has deep roots to get water and can tolerate the salty environment.
- Lichens (see Figure 1, next page) are organisms usually made up of a fungus and an alga. They're able to survive in rocky conditions because the fungus secretes acids which erode the rock, releasing minerals.
- Shrubs of the *Calligonum* genus are pioneer species that can grow in areas that experience periodic drought.

Pioneer species change the abiotic conditions — they die and microorganisms decompose the dead organic material (humus), which forms a basic soil. This makes conditions less hostile, e.g. the basic soil helps to retain water, so new organisms with different adaptations can move in and grow.

The new organisms then die and are decomposed, adding more organic material, making the soil deeper and richer in minerals such as nitrates. Nitrogen-fixing bacteria turn nitrogen from the atmosphere into ammonia. This forms ammonium ions in solution that can then be used by plants (see page 303). This means larger plants like shrubs can start to grow in the deeper soil, which retains even more water and contains more nutrients.

Some new species may change the environment so that it becomes less suitable for the previous species. For example, sand sedge stabilises the sand through the growth of rhizomes (underground stems). This makes the conditions less suitable for marram grass, which needs constant reburial by sand in order to grow healthily.

Later stages of succession

At each stage, different plants and animals that are better adapted for the improved conditions move in, out-compete the plants and animals that are already there, and become the dominant species in the ecosystem. The dominant species are the ones which cause the most change to the abiotic environment, making it more suitable for other species.

As succession goes on, the ecosystem becomes more complex. New species move in alongside existing species, which means that biodiversity increases. Plants create more habitats for animals, the abiotic conditions become less hostile and the amount of biomass increases.

Eventually these changes result in a **climax community** — the ecosystem is supporting the largest and most complex community of plants and animals it can. It won't change much more — it's in a steady state.

Tip: You learnt about biodiversity in the first year of your course — it's the variety of living organisms in an area.

Tip: Biomass is the mass of living material in an ecosystem.

Tip: A community is all the populations of different species found in a habitat — see page 418.

Example

1. Bare rock lacks soil, is exposed to strong winds and has periods of drought. Lichens (the pioneer species) are able to survive because they can grow in cracks to avoid the wind, break down rock to release minerals and are adapted to survive periods of drought.



2. The lichens die and are decomposed helping to form a thin soil, which thickens as more organic material is formed. This means other species such as mosses can grow.



3. Larger plants that need more water can move in as the soil deepens, e.g. grasses and small flowering plants. The soil continues to deepen as the larger plants die and are decomposed.

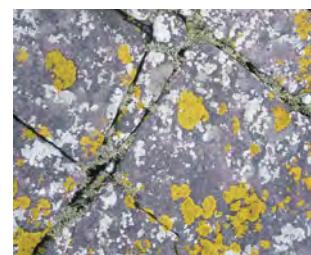


Figure 1: Lichens (orange and white) have adaptations that allow them to live on bare rock.

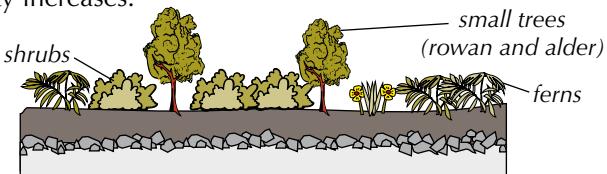
Tip: Primary succession also happens on sand dunes, salt marshes and even in lakes.

Exam Tip

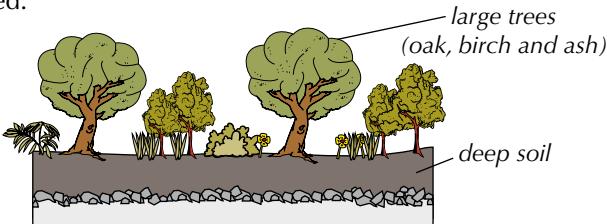
You don't need to learn the exact details of this example for the exam, but you do need to understand what's going on and why.

Tip: Tall plants can reduce the light available to shorter plants and can help stabilise fluctuating temperatures. For example, trees increase air humidity, provide shade and reduce wind, which moderates local temperatures.

- Shrubs, ferns and small trees begin to grow, out-competing the grasses and smaller plants to become the dominant species. Diversity increases.



- Finally, the soil is deep and rich enough in nutrients to support large trees. These become the dominant species, and the climax community is formed.



Tip: The main difference between the two types of succession is that soil is present at the start of secondary succession but not in primary succession. Secondary succession therefore tends to reach the climax community more quickly as a result.

Secondary succession

Secondary succession happens on land that's been cleared of all the plants, but where the soil remains, e.g. after a forest fire or where a forest has been cut down by humans. The established community of species is usually destroyed, but without too much disturbance to the soil. It can occur during any stage (including the climax community) after the pioneer stage.

The process of secondary succession is similar to primary succession, but because there's already a soil layer, secondary succession starts at a later stage — and the pioneer species are larger plants, e.g. shrubs.

Human impacts on succession

Human activities can prevent succession, stopping a climax community from developing. When succession is stopped artificially like this the climax community is called a plagioclimax.

Example

A regularly mown grassy field won't develop shrubs and trees (woody plants), even if the climate of the ecosystem could support them.

The growing points of the woody plants are cut off by the lawnmower, so larger plants can't establish themselves.

The longer the interval between mowing, the further succession can progress and the more diversity increases. But with more frequent mowing, succession can't progress and diversity will be lower — only the grasses can survive being mowed. Mowing doesn't just affect plants, it can affect the wider biodiversity of the area. For example, removing the woody plants destroys habitats for insects, decreasing the number of insect species.

Tip: Allowing animals to graze on land has a similar impact to mowing on succession — see page 435.

Climatic climax communities

Which species make up the climax community depends on what the climate's like in an ecosystem. The climax community for a particular climate is called its **climatic climax**.

Examples

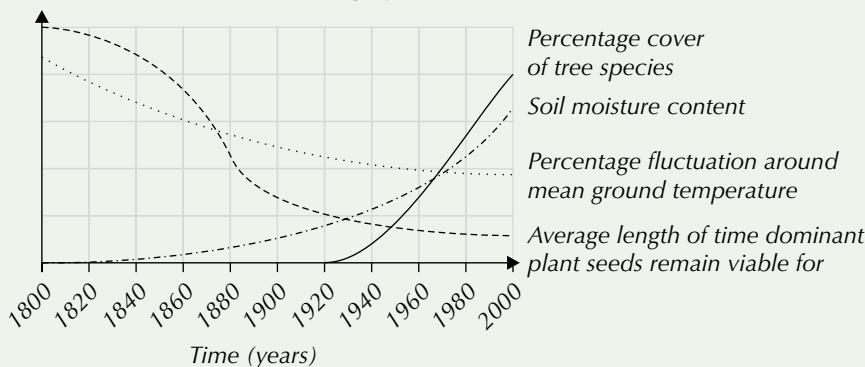
- In a temperate climate, e.g. the UK, there's plenty of available water, mild temperatures and not much change between the seasons. The climatic climax will contain large trees because they can grow in these conditions once deep soils have developed (see Figure 2).
- In a polar climate there's not much available water, temperatures are low and there are massive changes between the seasons. Large trees won't ever be able to grow in these conditions, so the climatic climax contains only herbs or shrubs, but it's still the climax community.



Figure 2: The climax community in many parts of Britain is deciduous woodland.

Practice Questions — Application

A team analysed data on ecological changes in part of a national park. Their results are shown in the graph below.



- What type of succession is shown on the graph? Explain your answer.
- Describe the characteristics of the dominant plant community between 1800 and 1860.
- Describe and suggest an explanation for the change shown in the average length of time dominant plant seeds remain viable for.
- During what time period would you expect to see a high percentage of plants whose seeds require high light intensity for germination? Explain your answer.
- Describe and suggest an explanation for the change in the soil moisture content shown on the graph.

Tip: 'Remains viable for' means how long the plant seeds are capable of germinating (sprouting).

Practice Questions — Fact Recall

- Why are ecosystems described as 'dynamic'?
- What is succession?
- Which type of succession happens in areas with no soil?
- Give an example of a pioneer species.
- What is a climax community?

Exam Tip

You need to be able to use the correct ecological terms (like primary succession, climax community, etc.) in your exam — and spell them correctly too.

Learning Objectives:

- Be able to show an understanding of the need to manage the conflict between human needs and conservation in order to maintain the sustainability of natural resources.
- Understand that conservation of habitats frequently involves management of succession.

Specification Reference 3.7.4

Tip: Remember, an ecosystem is all the organisms living in a community, plus all the abiotic conditions in the area in which they live (see page 415).

Tip: The method used to manage succession always depends on the environment, e.g. clearing winter scrub (grasses and low shrubs) that would otherwise build up and dry out the land is useful to prevent wetland becoming woodland.

Tip: There are lots of ways of managing succession to aid conservation, some of which involve altering the abiotic conditions. For example, ditches and sluices can be used to control the water content of the soil.

5. Conservation

Lots of things humans do endanger species and cause the loss of habitats. Happily, all is not lost — there are things we can do to protect them.

What is conservation?

Conservation is the protection and management of species and habitats (ecosystems) in a sustainable way. Sustainable means that enough resources are taken to meet the needs of people today, without reducing the ability of people in the future to meet their own needs.

Conservation is a dynamic process as conservation methods need to be adapted to the constant changes (caused naturally and by humans) that occur within ecosystems.

Conflicts in conservation

Not everyone agrees with every conservation measure though — there's often **conflict** between human needs and conservation. Careful management is needed to find a balance between the two and maintain the sustainability of natural resources.

Example

- The Maasai Mara is a national reserve in Kenya. It's a large area of grassland (savannah) with lots of wildlife.
- The Maasai people traditionally earn a living by raising livestock, such as cattle. This can bring them into conflict with conservationists — for example, overgrazing by livestock can destroy grassland for wildlife.
- Conservation trusts are working with the Maasai to help them make money from their land through conservation and ecotourism projects, as well as farming, and to farm in a sustainable way. So the economic needs of the Maasai are met, while still allowing the area and its wildlife to be conserved.

Conservation methods

Different species and habitats need to be conserved in different ways. Here are some examples of the different conservation methods that can be used.

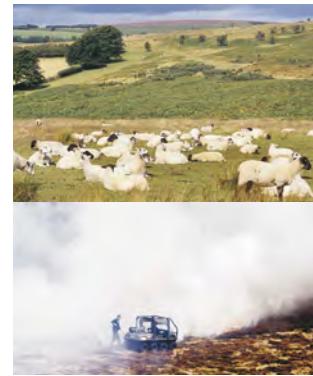
1. Management of succession

Human activities can interrupt the process of succession (see page 432). Conservation frequently involves preventing succession in order to preserve an ecosystem in its current stage of succession.

Example

There are large areas of moorland in Scotland that provide habitats for many species of plants and animals. If the moorland was left to natural processes, succession would lead to a climax community of spruce forest. This would mean the loss of the moorland habitat and could lead to the loss of some of the plants and animals that currently live there. Preventing succession keeps the moorland ecosystem intact. There are a couple of ways to manage succession to conserve the moorland ecosystem:

- Animals are allowed to graze on the land. This is similar to mowing — the animals eat the growing points of the shrubs and trees, which stops them from establishing themselves and helps to keep vegetation low.
- Managed fires are lit. After the fires, secondary succession will occur on the moorland — the plant species that grow back first (pioneer species) are the species that are being conserved, e.g. heather. Larger plant species will take longer to grow back and will be removed again the next time the moor's burnt.



2. Seed banks

A seed bank is a store of seeds from lots of different plant species. Seed banks act as a backup for the conservation of plant species in the wild — for example, if a plant species becomes extinct or is lost from a particular habitat, stored seeds can be used to reintroduce the species.

Seed banks are a good way of conserving plant species — large numbers of species can be conserved in a fairly small space as most seeds are quite small. Seeds can also be stored anywhere in the world and for long periods of time, as long as it's cool and dry. However, the seeds have to be regularly tested to see if they're still viable (whether they can grow into a plant), which can be expensive and time-consuming.

3. Captive breeding

Captive breeding programmes involve breeding animals in controlled environments. Species that are endangered, or already extinct in the wild, can be bred in captivity to help increase their numbers. However, there are some problems with captive breeding programmes, e.g. animals like pandas can have problems breeding outside their natural habitat, which can be hard to recreate in a zoo.

Animals bred in captivity can be reintroduced to the wild. This increases their numbers in the wild, which can help to conserve their numbers or bring them back from the brink of extinction. Reintroducing animals into the wild can cause problems though, e.g. reintroduced animals could bring new diseases to habitats, harming other species living there.

4. Fishing quotas

Fishing quotas are limits to the amount of certain fish species that fishermen are allowed to catch. Fishing quotas help to conserve fish species by reducing the numbers that are caught and killed — they aim to prevent a situation where fish populations reach such low levels that they are threatened with extinction.

However, fishing quotas can be unpopular with fishermen as they limit their potential income. There can also be problems with 'discards' — ships catching more fish than they are allowed to, then throwing back some fish (which are often dead already) so they don't exceed their quota. This is wasteful and doesn't contribute to the conservation of fish populations. In areas where there are fishing vessels from more than one country, international cooperation is needed for quotas to be fully effective.

5. Protected areas

Protected areas, like national parks and nature reserves, protect habitats and the species in them by restricting urban development, industrial development and farming. Habitats in protected areas can be managed to conserve them.

Tip: Fishing is a good example of an area where there is a conflict between human needs (our need for food and the need of fishermen to earn a living) and conservation needs. To protect fish populations for the future, we need to find ways to make fishing sustainable.

Exam Tip

The only conservation method you need to know about is managing succession. However, it's a good idea to be aware of other methods because you could be given data about them in the exam.

Tip: To answer these questions, you might need to take a look back at pages 430-432 to remind yourself about how succession works.

Exam Tip

You always need to read exam questions carefully, but that's especially true when you're given data on a topic you know really well. It's dead easy to give a general answer based on what you know when actually the question wants you to use the data you're given.

Tip: When a question says 'suggest' you're not expected to know the exact answer — you're expected to use your knowledge to come up with a sensible answer.

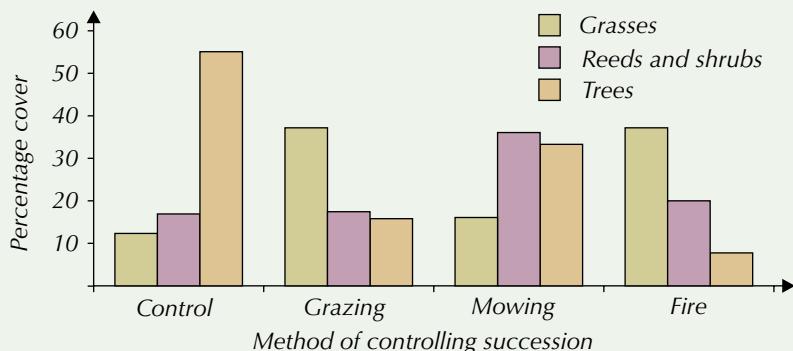
Example

Some woodlands are managed by coppicing — cutting down trees in a way that lets them grow back, so they don't need to be replanted. This helps to conserve the woodland, but allows some wood to be harvested.

Protected areas also have problems. For example, national parks are also used as tourist destinations (many are funded by revenue from tourists). This means there's conflict between the need to conserve the habitats and the need to allow people to visit and use them.

Practice Questions — Application

Succession in an area of steppe (grassland) can result in a forest. A nature reserve wishes to conserve the steppe landscape by managing succession. Grazing, mowing and fire were used on three areas that were then left for a set amount of time. The results were compared to a control area which was left undisturbed for the same length of time. The data is shown on the bar chart below.



- Q1
 - a) Describe what the results show about the effectiveness of the three methods of managing succession.
 - b) Suggest two advantages of controlling succession by grazing rather than by fire.
- Q2 The saiga antelope is an endangered species living in this area. A major cause of the population decline of the saiga has been hunting by local people for meat and also for its horns, which are sold for use in traditional medicine. Suggest why education programs and developing alternative livelihoods for local people could be a useful part of the effort to conserve the saiga antelope.

Practice Questions — Fact Recall

- Q1 What is conservation?
- Q2 Give one reason why people don't always agree with conservation measures.
- Q3 Describe how a managed fire can be used to prevent succession, in order to preserve an ecosystem in its current stage.
- Q4 Give an example of a method of conservation other than the management of succession.

6. Conservation Evidence and Data

You need to be able to evaluate data on conservation. Sometimes though, it can be a bit tricky — especially when data sets show conflicting trends...

Evaluating evidence on conservation

You need to be able to evaluate any evidence or data about conservation projects and research that the examiners throw at you — so here's an example I made earlier...

Learning Objective:

- Be able to evaluate evidence and data concerning issues relating to the conservation of species and habitats and consider conflicting evidence.

Specification Reference 3.7.4

Example

In recent years, native British bluebells have become less common in woodland areas. It's thought that this is due to the presence of non-native Spanish bluebells, which compete with the native species for a similar niche and are capable of breeding with the native species to produce a hybrid.

An experiment was carried out to see if removing the invasive Spanish species would help to conserve the native species. Each year for 15 years the percentage cover of native species was estimated in a 50 m by 50 m area of woodland using random sampling and 250, 1 m² quadrats. After five years, all the Spanish bluebells were removed. A similar sized control woodland in which the Spanish bluebells remained untouched was also studied. The results are shown below.

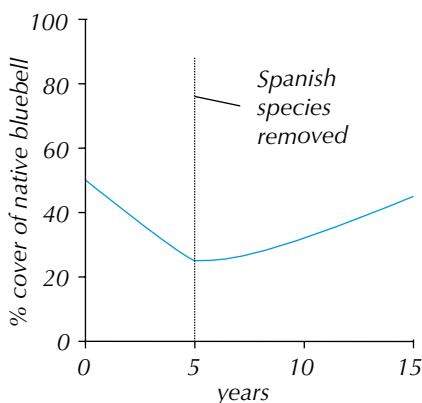


Figure 1: Percentage cover of native British bluebells in a woodland.

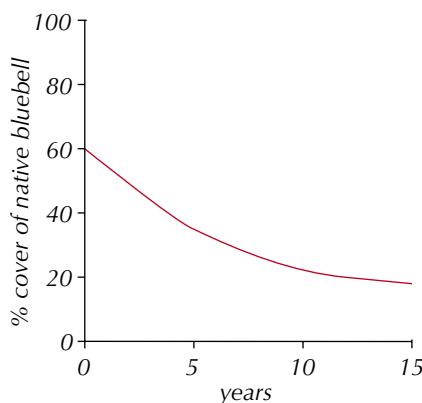


Figure 2: Control experiment.

Tip: A niche is the role of a species within its habitat (see page 415). If you need a reminder about using quadrats, take a look at page 424.



Figure 3: Some attempts to conserve British bluebells (top) have involved removing Spanish bluebells (bottom).

You might be asked to:

Describe the data

- For the first five years, the percentage cover of native bluebells fell from 50% to around 25%. After the Spanish species was removed, it increased from around 25% to around 45% in ten years.
- The control experiment shows a fairly steady drop in native bluebell percentage cover from 60% to 20% over the 15 years.

Draw conclusions

The removal of Spanish bluebells resulted in an increase in the percentage cover of native bluebells over a ten year period. This suggests that the recent decrease in native British bluebells is due to competition with the Spanish bluebells.

Evaluate the method

- The effects of some other variables (e.g. changing weather) were removed by the control experiment, where the percentage cover of native bluebells continued to fall throughout the 15 year study. This makes the test more valid.
- The study area and sample size were quite large, giving more accurate data.
- Random sampling removed bias — the data's more likely to be an accurate estimate of the whole area.

There's more about interpreting data and evaluating experiments on pages 15-17.

Tip: Ecosystems are such complicated things that studies can sometimes throw up conflicting data — which can be both really interesting and a bit of pain if the data for both studies looks precise. But the first thing you should always do when you get conflicting data is look at the methodology — chances are something in the method has caused the conflict.

Conflicting evidence

The evidence from one study alone wouldn't usually be enough to conclude that there's a link between decreasing percentage cover of native bluebells, and the presence of Spanish bluebells. Similar studies would be carried out to investigate the link.

If these studies came to the same conclusion, the conclusion would become increasingly accepted. Sometimes studies come up with conflicting evidence though — evidence that leads to a different conclusion than other studies.

Example

Another study was carried out to investigate the effect on native bluebells of removing Spanish bluebells.

It was similar to the study on the previous page except a 20 m by 20 m area was sampled using a random sample of 20 quadrats, and no control woodland was used.

You might be asked to:

Describe the data

In the first five years, the percentage cover of native bluebells fell from 50% to around 25%. After the Spanish species was removed, it kept decreasing to around 15% after the full 15 years.

Draw conclusions

The removal of the Spanish bluebells had no effect on the decreasing percentage cover of native bluebells — which conflicts with the study on the previous page.

Evaluate the method

- There wasn't a control woodland, so the continuing decrease in native bluebell cover after the removal of the Spanish bluebells could be due to another factor, e.g. cold weather in years 5-10.
- The study area and sample size were quite small, giving a less accurate total percentage cover.

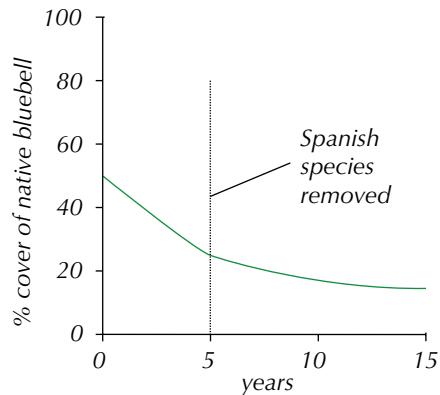
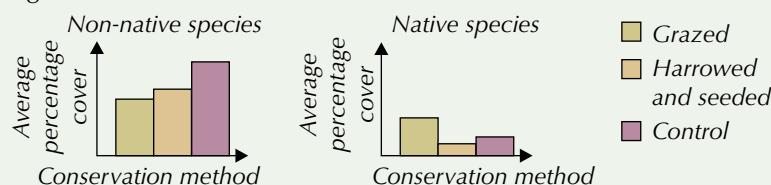


Figure 4: Percentage cover of native British bluebells in a woodland.

Practice Questions — Application

The diversity of native plants on certain areas of grassland has been reduced by the invasion of non-native species. A team wishing to conserve the native species investigated two methods. The first involved harrowing the soil and dispersing native seeds, and the second involved continual grazing by sheep. These methods were each conducted on 14 fields, and their effects were compared to a control field (which was left untouched). The fields were left for four years. The results were averaged and are shown below.



- Q1 Describe the results shown in the graph.
- Q2 a) Which method of conservation was most successful?
Give a reason for your answer.
- b) Suggest why the method you named in part a)
worked and why the other did not.
- Q3 A second team conducted an investigation using the same methods.
Each method was used on a single field and the fields were left for
six years.
- a) A different number of fields were used in the investigations
carried out by the two teams. Explain what effect this
would have on the validity of the results produced.
- b) Did the first investigation use a positive or
negative control? Explain your answer.

Tip: Harrowing is where the surface of the soil is broken up and smoothed over. This makes the soil better for seed growth.

Tip: If you need a reminder about the different types of control see page 2.

Section Summary

Make sure you know:

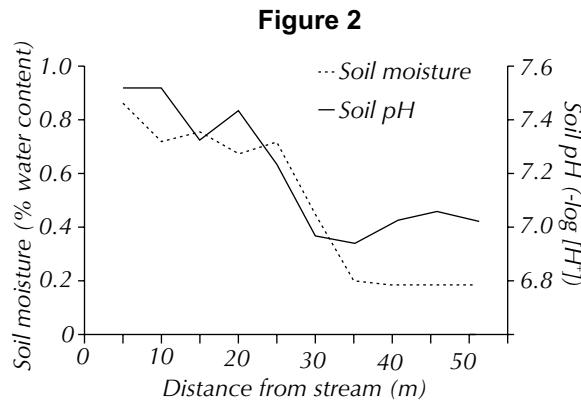
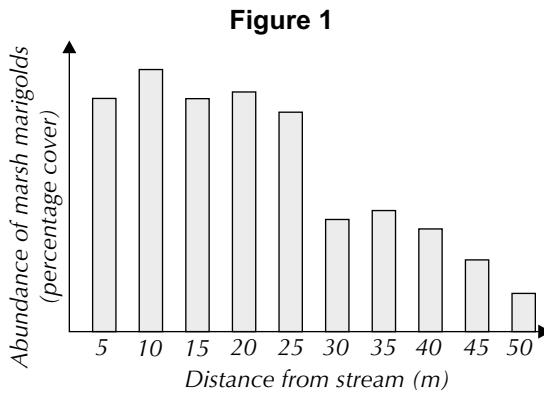
- That an ecosystem is all the organisms living in a community, plus all the abiotic conditions in the area in which they live.
- That ecosystems vary in size and can be very small, e.g. a pond, or very large, e.g. an entire ocean.
- That the place where an organism lives is known as its habitat.
- That a niche is the role of a species within its habitat and includes its biotic and abiotic interactions. Every species occupies its own unique niche.
- That species have adaptations that increase their chance of survival and reproduction, and that they are adapted to both the abiotic and biotic conditions in their niche.
- That a population is all the organisms of one species in a habitat and that the populations of different species in a habitat form a community.
- That the carrying capacity is the maximum stable population size of a species that an ecosystem can support.
- That population size can vary due to abiotic factors, e.g. the amount of light or space available.
- That population size can also vary because of biotic factors including interspecific competition, intraspecific competition and predation.

- That interspecific competition is when organisms of different species compete with each other for the same resources, and that it results in the less well-adapted species being out-competed.
- That intraspecific competition is when organisms of the same species compete with each other for the same resources, and results in a cyclical change in population size around an ecosystem's carrying capacity.
- That predation is when an organism (the predator) kills and eats another organism (the prey), and that this results in the population sizes of the predators and prey being interlinked.
- How to estimate the size of a population of slow-moving or non-motile organisms using randomly placed quadrats.
- How to use quadrats along a belt transect to investigate how the distribution of slow-moving or non-motile organisms changes across an area.
- How to estimate the population size of motile organisms using the mark-release-recapture method, and be able to calculate an estimate of population size from given data using this method.
- That the accuracy of the mark-release-recapture method depends on a few assumptions. These include that the marked sample has had enough time and opportunity to remix with the population, that marking doesn't affect the chances of survival of marked organisms, and that there are no changes in population size during the period of the study.
- How to investigate the effect of an environmental factor on the distribution of a species, e.g. the effect of soil pH on marram grass (Required Practical 12).
- That ecosystems are dynamic systems, which means they are constantly changing. The process by which this change occurs is called succession.
- That primary succession starts with pioneer species colonising a new land surface — pioneer species are so called because they are the first species to colonise an area.
- That succession is made up of stages. At each stage different plant and animal communities develop. Certain species can affect the environment (i.e. the abiotic and biotic conditions) so that it becomes more suitable for other species with different adaptations. As new species colonise an environment they may change it in a way that makes it less suitable for previous species. So as the process of succession takes place, communities of organisms are succeeded (replaced) by other communities.
- That during succession, organisms change their abiotic environment in ways that make the environment less hostile. This means that new species move in alongside existing species, which increases biodiversity.
- That a climax community is the largest and most complex community of plants and animals that an ecosystem can support. At this stage the ecosystem is in a steady state and won't change much.
- That humans can interrupt succession and stop a climax community from developing, e.g. by mowing, grazing or controlled burning — this keeps an ecosystem at a particular stage of succession.
- That conservation aims to manage species and habitats in a sustainable way in order to preserve natural resources, but this can create conflict with human needs. Conflicts require careful management in order to maintain the sustainability of natural resources.
- That the conservation of habitats frequently involves the management of succession by humans, e.g. allowing animals to graze on land. This is a method of conservation that preserves the ecosystem in its current stage of succession.
- That there are other methods to conserve species and habitats, which include the use of seed banks, captive breeding, fishing quotas and protected areas.
- How to evaluate evidence and data about conservation projects, including data from studies which give conflicting evidence.

Exam-style Questions

- 1 A team of scientists is investigating the distribution of marsh marigolds across a field that is directly next to a stream.
- 1.1 Suggest and describe a method the scientists could use to investigate the distribution of marsh marigolds. (2 marks)
- 1.2 The team decide they want to record the percentage cover of marsh marigolds. Describe how they could measure the percentage cover and give an advantage of measuring species abundance this way. (3 marks)
- 1.3 Abiotic factors were investigated at each place where data on marsh marigolds was recorded. Explain what is meant by the term 'abiotic conditions'. (1 mark)

The results of the investigation are recorded in **Figure 1** and **Figure 2**.



- 1.4 The team conclude that marsh marigolds grow better in waterlogged ground. How far does the data support this conclusion? Explain your answer. (5 marks)
- 1.5 The stream is prone to flooding and the land around it is often boggy. Describe **two** risks that the scientists should be aware of and suggest the appropriate course of action they should take to reduce their risk. (2 marks)
- 2 A student is investigating the population of a particular species of centipede (species Z) in his garden. He decides to use the mark-release-recapture method.
- 2.1 Describe how the student could use the mark-release-recapture method to estimate the population size of the species in his garden. (5 marks)
- 2.2 Use the data in **Table 1** to calculate the population size of species Z.

Table 1

| Individuals of species Z | Number of individuals in trap | |
|--------------------------|-------------------------------|---------------|
| | First sample | Second sample |
| Total caught | 10 | 15 |
| Total marked caught | - | 8 |

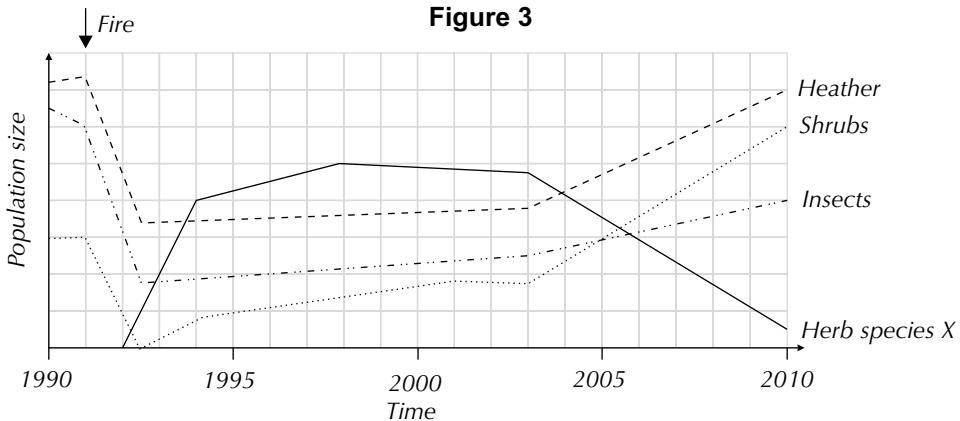
(1 mark)

- 3** An area of heathland in a national park is home to an endangered plant species which needs high light intensity and acidic soils to grow successfully. The park managers have decided to halt succession on the heath and are exploring ways in which to do this. They are considering burning the heathland every fifty years.

- 3.1** Suggest **two** reasons why burning the heathland every fifty years might not help to conserve the endangered plant species.

(2 marks)

The managers have found data from another heathland which halted succession by burning every 20 years. The data is shown in **Figure 3**.

Figure 3

- 3.2** Describe and explain the changes in the population sizes of heather, shrubs and insects between 1991 and 2000.

(3 marks)

- 3.3** Herb species X is a pioneer species.

Explain what is meant by the term 'pioneer species'.

(1 mark)

- 3.4** Describe the changes in the population size of herb species X shown in **Figure 3** over the twenty year period. Suggest explanations for the changes seen.

(3 marks)

- 3.5** The managers have met opposition against the use of fire to halt succession. Some campaigners against the use of fire have used the data shown above to argue that insect populations do not recover following heathland fires.

Suggest and explain **two** reasons why the data doesn't support this conclusion.

(2 marks)

- 3.6** Explain an alternative way in which the plant species could be conserved.

(3 marks)

1. Mutations

Genes are pretty awesome. However, their base sequences can sometimes be mutated, changing the protein that gets produced. You met mutations in Topic 4, but you need to know a bit more about them here.

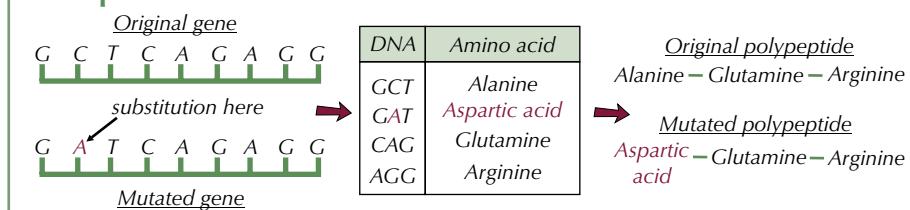
What are mutations?

Any change to the base (nucleotide) sequence of DNA is called a **mutation**. Mutations can be caused by errors during DNA replication. The rate of mutation can be increased by **mutagenic agents** (see page 446). The types of mutations that can occur include:

- **Substitution** — one or more bases are swapped for another, e.g. ATGCCT becomes ATTCCT (G is swapped for T).
- **Deletion** — one or more bases are removed, e.g. ATGCCT becomes ATCCT (G is removed).
- **Addition** — one or more bases are added, e.g. ATGCCT becomes ATGACCT (A is added).
- **Duplication** — one or more bases are repeated, e.g. ATGCCT becomes ATGCCCCCT (CC is repeated).
- **Inversion** — a sequence of bases is reversed, e.g. ATGCCT becomes ATCCGT (GCC is reversed to CCG).
- **Translocation** — a sequence of bases is moved from one location in the genome to another. This could be movement within the same chromosome or movement to a different chromosome.

The order of DNA bases in a gene determines the sequence of amino acids in a particular polypeptide. If a mutation occurs in a gene, the sequence of amino acids in the polypeptide that it codes for could be changed.

Example



Polypeptides make up proteins. A change in the amino acid sequence of a polypeptide may change the tertiary structure (final 3D shape) of the protein, which could mean that it doesn't work properly.

Example

A mutation in a polypeptide that makes up an enzyme may change the shape of the enzyme's active site. This may stop substrates from being able to bind to the active site, leaving the enzyme unable to catalyse the reaction.

Some mutations can increase the likelihood of developing certain cancers, e.g. mutations of the gene BRCA1 can increase the chances of developing breast cancer.

Learning Objectives:

- Know that gene mutations might arise during DNA replication and that they can include substitution, deletion, addition, duplication, inversion and translocation of bases.
- Know that mutations can result in a different amino acid sequence in the encoded polypeptide.
- Know that some gene mutations change only one triplet code and that, due to the degenerate nature of the genetic code, not all such mutations result in a change to the encoded amino acid.
- Understand that some gene mutations change the nature of all base triplets downstream from the mutation — they result in a frameshift.
- Be able to relate the nature of a gene mutation to its effect on the encoded polypeptide.

Specification Reference 3.8.1

Tip: Remember, when more than two amino acids join together, they form a polypeptide chain. The sequence of amino acids in the chain forms the primary structure of a protein and the final folding of the chain forms the tertiary structure.

Tip: Cystic fibrosis is a genetic disorder of the cell membranes. It results in the body producing a lot of thick sticky mucus in the air passages and in the pancreas.

Tip: Not all hereditary mutations are harmful — beneficial hereditary mutations drive evolution (see p. 405).

Tip: If a mutation doesn't cause a change in the amino acid order, it's called a 'silent mutation'.

Some mutations can cause **genetic disorders** — inherited disorders caused by abnormal genes or chromosomes, e.g. cystic fibrosis.

If a gamete (sex cell) containing a mutation for a type of cancer or a genetic disorder is fertilised, the mutation will be present in the new fetus formed — these are called **hereditary mutations** because they are passed on to the offspring.

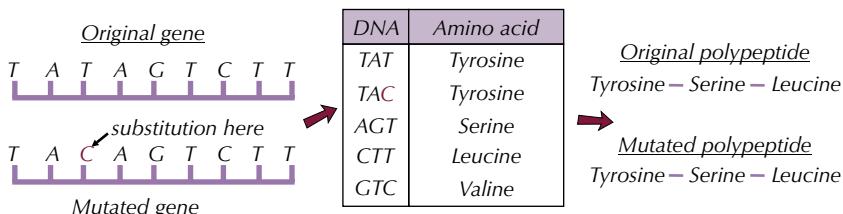
Mutations and proteins

Not all mutations affect the order of amino acids in a protein. The degenerate nature of the genetic code means that some amino acids are coded for by more than one DNA triplet (e.g. tyrosine can be coded for by TAT or TAC in DNA). This means that not all types of mutation will always result in a change to the amino acid sequence of the polypeptide.

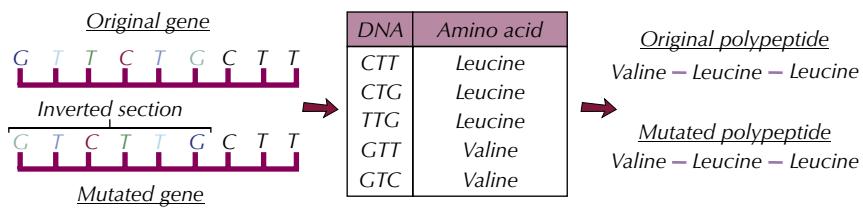
Examples

Some substitutions will still code for the same amino acid.

In this example, the mutated polypeptide is the same as the original polypeptide, despite the base substitution.



Sometimes, inversion mutations don't cause a change in the amino acid sequence either. In this example, the mutated polypeptide is the same as the original polypeptide, despite the reversal of the bases.

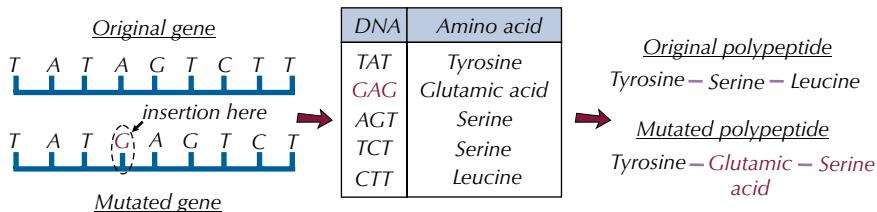


Frameshift mutations

Some mutations have a huge effect on the base sequence of a gene. Additions, duplications and deletions within a gene will almost always change the amino acid sequence of a polypeptide. That's because these mutations all change the number of bases in the DNA code. This causes a shift (called a **frameshift**) in the base triplets that follow, so that the triplet code is read in a different way.

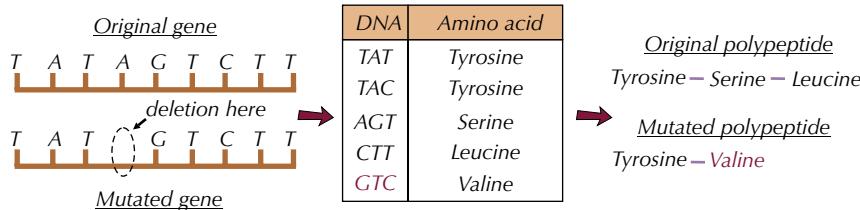
Examples

Here's how an addition can cause a frameshift and change the amino acid order:



Exam Tip
You could be asked to predict how much a mutation will affect a protein's structure. Just remember that a frameshift mutation affects more amino acids than a substitution mutation, so it will have a bigger overall effect on the protein's structure.

Here's how a deletion can cause a frameshift and change the amino acid order:



Tip: The base triplets that follow on from the mutation are said to be 'downstream' of the mutation.

Practice Questions — Application

The table below shows some amino acids and the base triplets that code for them.

| Base Triplet(s) | Amino Acid |
|-----------------|------------|
| GAT | Asp |
| CAT | His |
| ATA | Ile |
| CTT/CTC | Leu |
| ATG | Met |
| ACA | Thr |
| TAT | Tyr |

The following letters represent part of the DNA base sequence of a gene:

CTTCATGATACA

Look at the four mutated base sequences below.

Mutation A: CTCCATGATACA

Mutation B: CTTCATCATACA

Mutation C: CTTATGATACA

Mutation D: CTTCTTCATGATACA

Q1 For each of the base sequences:

- State the type of mutation that has taken place.
- Give the amino acid sequence coded for by the mutated gene.

Q2 Explain which mutation is likely to have:

- the least serious effect on the structure of the protein produced,
- the most serious effect on the structure of the protein produced.

Tip: 'Asp', 'His', 'Ile', etc are just abbreviated names of amino acids, e.g. 'Asp' is short for aspartic acid.

Practice Questions — Fact Recall

Q1 What is a mutation?

Q2 Briefly describe what happens in a translocation mutation.

Q3 Explain why a mutation in a polypeptide that makes up an enzyme could affect the enzyme's function.

Q4 What is a hereditary mutation?

Q5 Do mutations always affect the protein coded for by a gene?

Explain your answer.

Exam Tip

If you're asked how a mutation affects protein structure in the exam, don't fall into the trap of only writing about how the mutation will change the base sequence. Make sure you make it clear how the altered base sequence will affect both the amino acid sequence and the protein's structure.

Learning Objective:

- Understand that gene mutations occur spontaneously and that the mutation rate is increased by mutagenic agents.

Specification Reference 3.8.1

Tip: Remember, DNA replicates itself every time a cell divides.



Figure 1: The circled chromosomes have been damaged by exposure to an alkylating agent.

2. Mutagenic Agents

Mutations just happen, but there are certain things that can make them happen more often...

What are mutagenic agents?

Mutations occur spontaneously, e.g. when DNA is misread during replication. But some things can increase the rate of mutations — these are called **mutagenic agents**. Ultraviolet radiation, ionising radiation, some chemicals and some viruses are examples of mutagenic agents. They can increase the rate of mutations in different ways.

1. Acting as a base

Chemicals called base analogs can substitute for a base during DNA replication, changing the base sequence in the new DNA.

Example

5-bromouracil is a base analog that can substitute for thymine. It can pair with guanine (instead of adenine), causing a substitution mutation in the new DNA.

2. Altering bases

Some chemicals can delete or alter bases.

Example

Alkylating agents can add an alkyl group to guanine, which changes the structure so that it pairs with thymine (instead of cytosine).

3. Changing the structure of DNA

Some types of radiation can change the structure of DNA, which causes problems during DNA replication.

Example

UV radiation can cause adjacent thymine bases to pair up together.

Practice Questions — Application

- Q1 2-aminopurine is a base analog that can substitute for both adenine and guanine during DNA replication.
Explain why 2-aminopurine is a mutagenic agent.
- Q2 Mustard gas is an alkylating agent, sometimes used in chemical warfare. Exposure to it results in severe burns and blisters.
Explain how exposure to mustard gas may affect the sequence of amino acids in a particular polypeptide.

Practice Questions — Fact Recall

- Q1 When might a mutation occur spontaneously?
Q2 What is a mutagenic agent?
Q3 Give an example of a mutagenic agent.

3. Cancer

If a mutation occurs in a gene that controls cell division it can cause cancer. This is because the genes that control cell division don't behave as they should, which can result in uncontrolled cell growth.

Cell division and cancer

Mutations that occur in individual cells after fertilisation (e.g. in adulthood) are called **acquired mutations**. If these mutations occur in the genes that control the rate of cell division (by mitosis), it can cause uncontrolled cell division. If a cell divides uncontrollably the result is a **tumour** — a mass of abnormal cells. Tumours that invade and destroy surrounding tissue are called **cancers** (see next page).

There are two types of gene that control cell division — **tumour suppressor genes** and **proto-oncogenes**. Mutations in these genes can cause cancer.

Tumour suppressor genes

When functioning normally, tumour suppressor genes slow cell division by producing proteins that stop cells dividing or cause them to self-destruct (apoptosis) — see Figure 1.

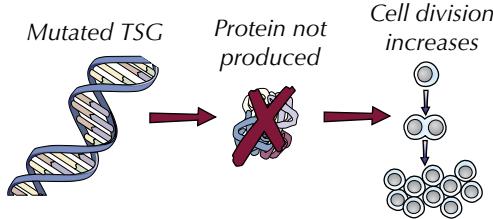


Figure 2: A mutated tumour suppressor gene (TSG) results in uncontrolled cell division.

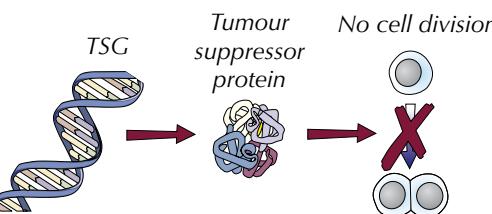


Figure 1: Action of a normal tumour suppressor gene (TSG).

If a mutation occurs in a tumour suppressor gene, the gene will be inactivated. The protein it codes for isn't produced and the cells divide uncontrollably (the rate of division increases) resulting in a tumour — see Figure 2.

Learning Objectives:

- Understand the role of tumour suppressor genes and oncogenes in the development of tumours.
- Know the main characteristics of benign and malignant tumours.
- Understand the role of abnormal methylation of tumour suppressor genes and oncogenes in the development of tumours.
- Understand the role of increased oestrogen concentrations in the development of some breast cancers.

Specification Reference 3.8.2.3

Tip: Apoptosis is a type of programmed cell death. It's where cells that are infected, damaged or have reached the end of their functional life are destroyed.

Proto-oncogenes

When functioning normally, proto-oncogenes stimulate cell division by producing proteins that make cells divide — see Figure 3.

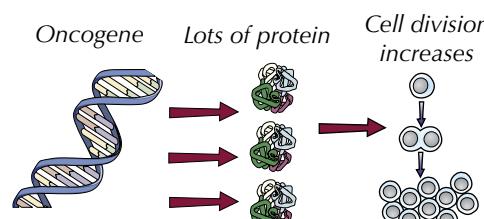


Figure 4: A mutated proto-oncogene stimulates uncontrolled cell division.

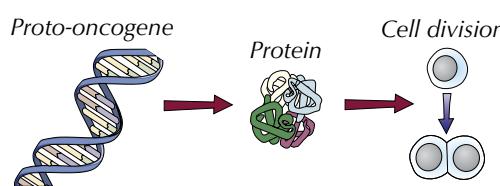


Figure 3: Action of a normal proto-oncogene.

If a mutation occurs in a proto-oncogene, the gene can become overactive. This stimulates the cells to divide uncontrollably (the rate of division increases) resulting in a tumour — see Figure 4. A mutated proto-oncogene is called an **oncogene**.

Tip: Mutations in tumour suppressor genes and proto-oncogenes are often acquired but some are inherited.

Tip: If you're struggling to remember which gene does what, the clue is in the name. Tumour suppressor genes **suppress** the growth of tumours — so they're the ones that slow down cell division.

Tumours and cancers

Tip: You met the lymphatic system in Topic 3. It's a network of tubes which transports excess tissue fluid back into the circulatory system.

Tip: You need to understand the difference between benign and malignant. You should never say benign cancers — there's no such thing. Only malignant tumours are cancerous.



Figure 5: MRI scan showing a benign brain tumour (top right).

Tumours can develop for years without any obvious symptoms and can be quite large by the time they're discovered. Not all tumours are cancerous — there are two different types:

1. Malignant tumours

Malignant tumours are cancers. They usually grow rapidly and invade and destroy surrounding tissues. Cells can break off the tumours and spread to other parts of the body in the bloodstream or lymphatic system.

2. Benign tumours

Benign tumours are not cancerous. They usually grow slower than malignant tumours and are often covered in fibrous tissue that stops cells invading other tissues. Benign tumours are often harmless, but they can cause blockages and put pressure on organs. Some benign tumours can become malignant.

Tumour cells

Tumour cells can differ from normal cells in many different ways:

- The nucleus is larger and darker than in normal cells. Sometimes the cells have more than one nucleus.
- They have an irregular shape.
- They don't produce all the proteins needed to function correctly.
- They have different antigens on their surface.
- They don't respond to growth regulating processes.
- They divide (by mitosis) more frequently than normal cells.

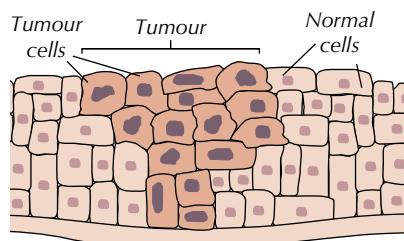


Figure 6: The appearance of tumour cells in comparison to normal cells.

Example

Figure 7 below shows a light micrograph of a culture of normal cells. Figure 8 shows the same cells following exposure to a cancer-causing chemical. The cells in Figure 8 are a lot denser, are a lot darker and have a more irregular structure.

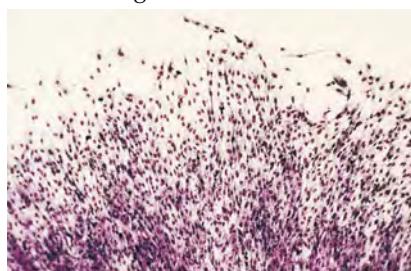


Figure 7: Light micrograph of a culture of normal cells.



Figure 8: Light micrograph of cells shown in Figure 7, following exposure to a cancer-causing chemical.

Causes of tumour growth

There are a number of different factors that are thought to lead to the growth of tumours. These include abnormal methylation of DNA and increased exposure to oestrogen.

Abnormal methylation

Methylation means adding a methyl ($-CH_3$) group onto something. Methylation of DNA is an important method of regulating gene expression — it can control whether or not a gene is **transcribed** (copied into mRNA) and **translated** (turned into a protein). When methylation is happening normally, it plays a key role in many processes in the body.

It's only when it happens too much (**hypermethylation**) or too little (**hypomethylation**) that it becomes a problem. The growth of tumours can be caused by abnormal methylation of certain cancer-related genes.

Tip: You should remember transcription and translation from Topic 4. The regulation of these processes is covered in more detail on pages 461-463.

Examples

- When tumour suppressor genes are hypermethylated, the genes are not transcribed — so the proteins they produce to slow cell division aren't made. This means that cells are able to divide uncontrollably by mitosis and tumours can develop.
- Hypomethylation of proto-oncogenes causes them to act as oncogenes — increasing the production of the proteins that encourage cell division. This stimulates cells to divide uncontrollably, which causes the formation of tumours.

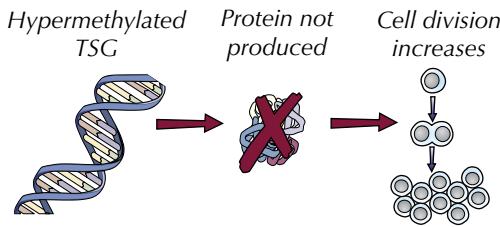


Figure 9: The result of hypermethylation of a tumour suppressor gene (TSG).

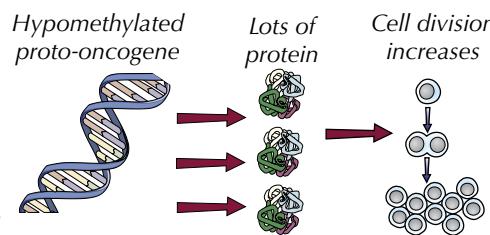


Figure 10: The result of hypomethylation of a proto-oncogene.

Tip: You can read more about methylation on page 466.

Tip: Make sure that you don't get 'hypermethylation' and 'hypomethylation' mixed up. Remember — 'hypo' means low.

Role of oestrogen in breast cancer

Some women may be exposed to more oestrogen than others. Increased exposure to oestrogen may be the result of starting menstruation earlier than usual, starting the menopause later than usual, or taking oestrogen-containing drugs, such as HRT.

Increased exposure to oestrogen over an extended period of time is thought to increase a woman's risk of developing breast cancer. The exact reasons behind this aren't fully understood, but there are a few theories as to how oestrogen can contribute to the development of some breast cancers:

- Oestrogen can stimulate certain breast cells to divide and replicate. The fact that more cell divisions are taking place naturally increases the chance of mutations occurring, and so increases the chance of cells becoming cancerous.
- Oestrogen's ability to stimulate division could also mean that if cells do become cancerous, their rapid replication could be further assisted by oestrogen, helping tumours to form quickly.
- Other research suggests that oestrogen is actually able to introduce mutations directly into the DNA of certain breast cells, again increasing the chance of these cells becoming cancerous.

Tip: HRT stands for hormone replacement therapy. It is used to increase oestrogen (and usually also progesterone) levels in some women in order to treat symptoms experienced during the menopause (the end of menstruation).

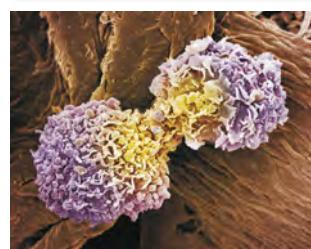


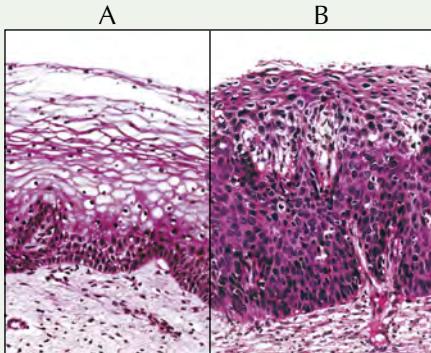
Figure 11: Micrograph of dividing breast cancer cells. Oestrogen is thought to stimulate this division, increasing the rate of tumour formation.

Practice Questions — Application

Exam Tip

If you're given a light micrograph to look at in the exam, don't panic — it's just a way of getting you to apply your knowledge. Read the question carefully to work out what it is that you're looking at. Then have a think about which part of your course it's testing and do a short mental recap of it — that should help you answer the question.

- Q1 Cervical intraepithelial neoplasia is a condition in which cells on the surface of the cervix grow abnormally. If untreated, these changes can lead to cancer of the cervix. One of the light micrographs below shows healthy cervical tissue. The other light micrograph shows tissue with cervical intraepithelial neoplasia.



Which of the light micrographs (A or B) do you think shows tissue with cervical intraepithelial neoplasia? Explain your answer.

- Q2 p53 is a tumour suppressor gene. Mutations in p53 are found in over half of all cancers.
- Suggest how p53 normally functions.
 - Suggest how mutations in p53 could lead to cancer.
- Q3 A woman has found a lump in her breast. After tests, her doctor tells her that she has breast cancer and that it has spread to her liver. The woman is 49 years old and is having oestrogen-containing HRT to treat symptoms of the menopause.
- What type of tumour does she have? Explain your answer.
 - Explain why taking HRT may have contributed to the woman developing breast cancer.
- Q4 Dichloroacetic acid is a carcinogen (cancer-causing chemical). Research has shown that dichloroacetic acid causes hypomethylation of c-myc (a proto-oncogene).
- Explain why dichloroacetic acid is a carcinogen.

Practice Questions — Fact Recall

- Q1 What is an acquired mutation?
- Q2 What is a mass of cells resulting from uncontrolled cell division known as?
- Q3 Describe the role of proto-oncogenes.
- Q4 What is an oncogene?
- Q5 Give a difference between a benign tumour and a malignant tumour.
- Q6 Explain how hypermethylation of tumour suppressor genes can cause a tumour to develop.
- Q7 Give one way in which increased exposure to oestrogen over a period of time is thought to contribute to the development of some breast cancers.

4. Interpreting Data on Cancer

As you've probably gathered from reading the papers, there's lots of things that could 'give you cancer'. You need to be able to evaluate evidence of genetic and environmental risk factors for cancer...

Risk factors for cancer

There's no single cause for cancer but scientists have identified lots of different 'risk factors' — things that increase a person's chance of getting cancer. Risk factors can be either genetic or environmental.

Genetic factors

Some cancers are linked with specific inherited **alleles** (an allele is a version of a gene). If you inherit that allele you're more likely to get that type of cancer (but it doesn't mean you'll definitely get that type of cancer).

Example

Hereditary mutations of the gene BRCA1 can greatly increase the chance of a woman developing breast cancer in her lifetime.

Learning Objectives:

- Be able to evaluate evidence showing correlations between genetic and environmental factors and various forms of cancer.
- Be able to interpret information relating to the way in which an understanding of the roles of oncogenes and tumour suppressor genes could be used in the prevention, treatment and cure of cancer.

Specification Reference 3.8.2.3

Environmental factors

Exposure to radiation, lifestyle choices such as smoking, increased alcohol consumption, and a high-fat diet have all been linked to an increased chance of developing some cancers.

Interpreting the data on risk factors

Data on variation (the differences that exist between individuals) can be very tricky to interpret because some characteristics can be affected by many different genes (they're polygenic) and many environmental factors. It's difficult to know which factors (genes or environment) are having the greatest effect. This makes it hard to draw conclusions about the causes of variation.

Example

Take a look at Figure 1:

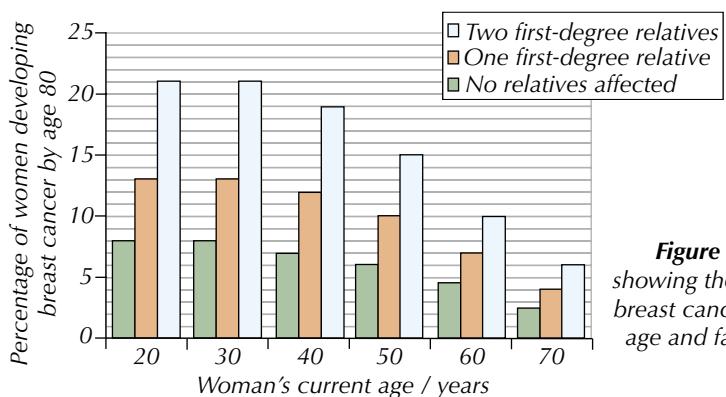


Figure 1: Graph showing the incidence of breast cancer defined by age and family history.

This graph shows how the incidence of breast cancer is affected by both age and family history. There's a **positive correlation** between incidence of breast cancer in women and the number of their first-degree relatives who have also had breast cancer.

The effect of family history decreases with age, but the incidence of breast cancer is always higher in women with a close family history of the disease. A woman is more likely to develop breast cancer if members of her family have had breast cancer, which suggests a **genetic link**.

Tip: A hereditary mutation in the BRCA1 gene might significantly increase your chances of developing breast cancer — but it doesn't mean you definitely will develop breast cancer.

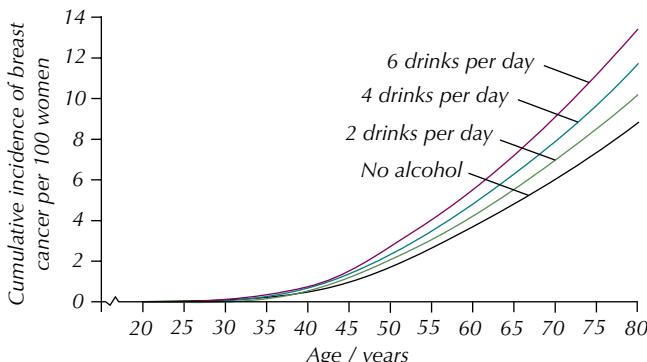
Tip: You can read more about variation on page 405.

Tip: First-degree relatives in this case include mothers, sisters and daughters.

Tip: A positive correlation means that as one variable increases so does the other. Have a look at page 15 for more on correlations.

Tip: Look carefully at titles, labels and scales on graphs. They're all there to help you understand the data.

Now look at Figure 2:



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http://www.nature.com/bjc/index.html

Figure 2: Graph showing the estimated incidence of breast cancer per 100 women according to the number of alcoholic drinks consumed each day.

This graph shows that the incidence of breast cancer is linked to both age and alcohol consumption. The graph shows that the incidence of breast cancer in women increases with age — i.e. there's a positive correlation between incidence of breast cancer and age.

There's also a positive correlation between the number of alcoholic drinks consumed each day and incidence of breast cancer. Alcohol consumption is an environmental factor.

Drawing conclusions from the evidence...

If you only saw one of these graphs you may think only genetics and age, or only alcohol consumption and age, affect your risk of developing breast cancer.

When you look at both sets of data you can see that all these things affect the risk. It's difficult to tell which factor (genes or alcohol) has the largest effect. Also, there are other environmental factors that are thought to be involved in increasing the risk of developing breast cancer (e.g. diet, exercise, etc.) that aren't considered here.

Exam Tip

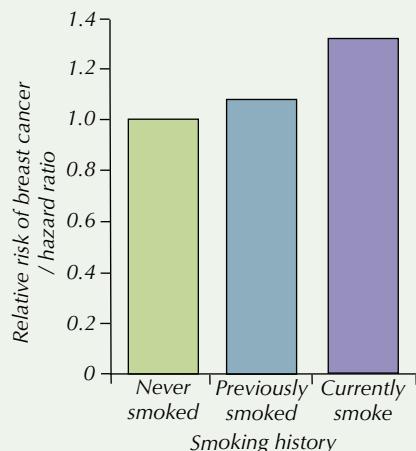
In the exam you might have to evaluate evidence showing correlations between genetic and environmental factors and cancer. Just remember that there are usually several factors at work and that correlation doesn't always mean cause.

Practice Question — Application

Q1 A study was carried out to determine if smoking is linked to an increased risk of breast cancer. 116 544 women without breast cancer in California were sent questionnaires to establish their smoking history and other personal information. The women were then followed for 5 years.

The results on the right show the relative risk of breast cancer, adjusted for other factors such as age and alcohol consumption, for women with different smoking histories.

- Describe the results shown in the graph.
- Can you conclude from this data that smoking causes breast cancer? Explain your answer.



Preventing, treating and curing cancer

Cancer is caused by mutations in proto-oncogenes and tumour suppressor genes (see page 447). Understanding the role that these genes play in causing cancer, and knowing exactly how they work, can be really helpful for coming up with ways to prevent, treat and cure cancer.

Preventing cancer

If a specific cancer-causing mutation is known, then it is possible to **screen** for (look for) the mutation in a person's DNA.

Example

It's possible to screen for the mutated allele of BRCA1 (the tumour suppressor gene, which greatly increases a woman's risk of developing breast cancer in her lifetime).

Knowing about this increased risk means that preventative steps can be taken to reduce it.

Example

A woman with the BRCA1 mutation may choose to have a mastectomy (removal of one or both breasts) to significantly reduce the risk of breast cancer developing. Women with this mutation may also be screened for signs of breast cancer more often than the rest of the population, as early diagnosis increases the chances of recovery.

Tip: It's very rare that such an extreme measure (like removing a breast) is taken, but a mutation in BRCA1 gives an extremely high (50-65%) chance of breast cancer developing.

Knowing about specific mutations also means that more sensitive tests can be developed, which can lead to earlier and more accurate diagnoses.

Examples

- There's a mutation in the RAS proto-oncogene in around half of all bowel cancers. Bowel cancer can be detected early by looking for RAS mutations in the DNA of bowel cells.
- People with a mutated APC tumour suppressor gene have frequent colonoscopies to diagnose hereditary colon cancer earlier.

Tip: A colonoscopy involves attaching a camera onto a long tube and passing it through the colon.



Figure 3: Colonoscopic view of a human colon with colon cancer. Tumours appear bright red.

Treating and curing cancer

The treatment for cancer can be different for different mutations, so knowing how specific mutations actually cause cancer can be very useful for developing drugs to effectively target them.

Examples

- Skin cancer caused by a mutation of the B-RAF proto-oncogene can be treated with the drug ZELBORAF™. ZELBORAF™ inhibits the mutated B-RAF enzyme — this stops cells that express the mutation from growing. Skin cancers caused by other mutations can't be treated this way.
- Breast cancer caused by a mutation of the HER2 proto-oncogene can be treated with a drug called Herceptin®. This drug binds specifically to the altered HER2 protein receptor and suppresses cell division and tumour growth. Breast cancer caused by other mutations is not treated with this drug as it doesn't work.
- Research is being conducted into a treatment for breast, pancreatic and cervical cancers caused by a faulty BRCA tumour suppressor gene. This involves using small molecules which block an enzyme involved in repairing DNA. The molecules may be able to prevent the DNA repair in cancerous cells containing a faulty BRCA gene.

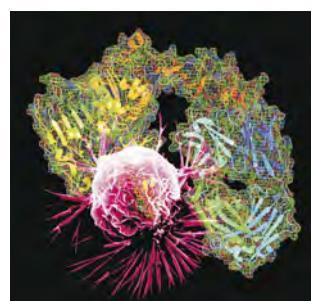


Figure 4: Molecular model of Herceptin® (green) and a breast cancer cell (pink).

This kills the cancer cells and so could provide a targeted treatment for cancers caused by BRCA mutations.

Some cancer-causing mutations require more aggressive treatment than others, so understanding how the mutation that causes them works can help produce the best treatment plan.

Example

If a mutation is known to cause an aggressive (fast-growing) cancer, it may be treated with higher doses of radiotherapy or by removing larger areas of the tumour and surrounding tissue during surgery.

Tip: Radiotherapy involves using radiation in an attempt to shrink a tumour. The radiation damages the DNA of the tumour cells, causing them to die and the tumour to shrink.

Gene therapy (where faulty alleles in a person's cells are replaced by working versions of those alleles — see page 487) may also be able to treat cancer caused by some mutations.

Example

If you know that the cancer is being caused by inactivated tumour suppressor genes, it's hoped that gene therapy could be used in the future to provide working versions of the genes.

Currently, gene therapy has only been used to treat cancer in clinical trials.

Tip: Remember, an acquired mutation is a mutation that occurs in individual cells after fertilisation (e.g. in adulthood). Hereditary mutations are mutations passed from a parent to their offspring via their gametes.

Tip: You need to know how tumour suppressor genes and proto-oncogenes work to answer Q2. Take a look back at p. 447 if you get stuck.

Tip: When doctors biopsy a tumour they take a small sample of cells from it to test and examine under a microscope.

Practice Questions — Application

Q1 Retinoblastoma is a rare form of childhood cancer, characterised by tumours in one or both eyes. It is caused by a mutation in the RB1 tumour suppressor gene, which codes for the pRB protein.

- The RB1 gene has two alleles. A mutation has to occur in both RB1 alleles for retinoblastoma to develop. Suggest why this is the case.
- Some children with retinoblastoma inherit a mutation in one of their RB1 alleles. These children are more likely to develop further tumours than children who acquire a mutation in early childhood.

Explain how screening to determine whether a child has inherited an RB1 mutation may be beneficial.

Q2 Imatinib is an anti-cancer drug that inhibits the function of CD117, a receptor protein produced by the KIT oncogene.

- The KIT oncogene is responsible for some gastrointestinal tumours. Suggest why a doctor will biopsy one of these tumours and test it for the presence of CD117 before deciding on a course of treatment.
- There are many anti-cancer drugs available that target oncogenes. They work by inhibiting the function of the oncogene protein. Developing a drug against mutated tumour suppressor genes can be more difficult. Suggest why this might be the case.

5. Stem Cells

All multicellular organisms stem from, err, stem cells. Every cell in your body was produced from a stem cell. So was every cell in every other multicellular organism's body. So they're pretty important.

What are stem cells?

Multicellular organisms are made up from many different cell types that are specialised for their function, e.g. liver cells, muscle cells, white blood cells. All these specialised cell types originally came from stem cells. Stem cells are unspecialised cells that can develop into other types of cell. Stem cells divide to become new cells, which then become specialised.

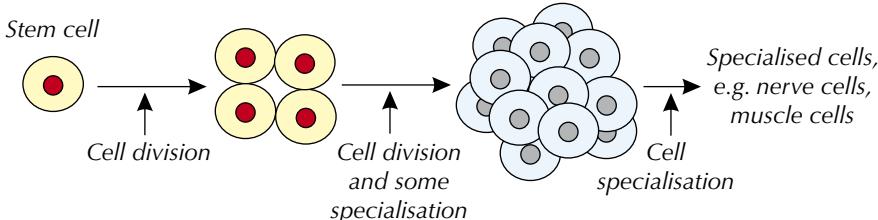


Figure 1: Diagram showing stem cell division.

Where are stem cells found?

All multicellular organisms have some form of stem cell. Stem cells are found in the embryo (where they become all the specialised cells needed to form a fetus) and in some adult tissues (where they become specialised cells that need to be replaced, e.g. stem cells in the intestines constantly replace intestinal epithelial cells).

Stem cells that can mature (develop) into any type of body cell in an organism, (including the cells that make up the placenta in mammals) are called **totipotent** cells. Totipotent stem cells are only present in mammals in the first few cell divisions of an embryo. After this point the embryonic stem cells become **pluripotent**. They can still specialise into any cell in the body, but lose the ability to become the cells that make up the placenta.

The stem cells present in adult mammals are either **multipotent** or **unipotent**. Multipotent stem cells are able to differentiate into a few different types of cell.

Example

Both red and white blood cells can be formed from multipotent stem cells found in bone marrow.

Unipotent stem cells can only differentiate into one type of cell.

Example

There's a type of unipotent stem cell that can only divide to produce epidermal skin cells, which make up the outer layer of your skin.

Becoming specialised

Stem cells become specialised because during their development they only transcribe and translate part of their DNA. Stem cells all contain the same genes — but during development not all of them are transcribed and translated (expressed). Under one set of conditions, certain genes are expressed and others are switched off. Under different conditions, different genes are expressed and others are switched off.

Learning Objectives:

- Know that totipotent cells are cells that can mature into any type of body cell and that they occur only for a limited time in mammalian embryos.
- Know that pluripotent cells are cells that can mature into any type of body cell (except placental cells) and that they occur in mammalian embryos.
- Know that multipotent and unipotent cells are found in mature mammals and that they can divide to form a limited number of different cell types.
- Understand that during development, totipotent cells translate only part of their DNA, resulting in cell specialisation.
- Know about unipotent cells, exemplified by the formation of cardiomyocytes.

Specification Reference 3.8.2.1

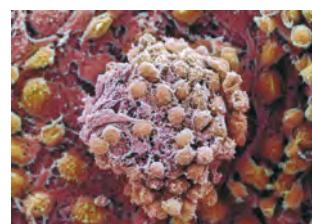


Figure 2: A cluster of human embryonic stem cells.

Tip: Remember: transcription is when DNA is copied into mRNA. Translation is when proteins are produced using the code in mRNA.

Tip: The process of cells becoming specialised is known as differentiation.

Tip: It's a mix of some genes being switched on and other genes being switched off that causes specialisation.

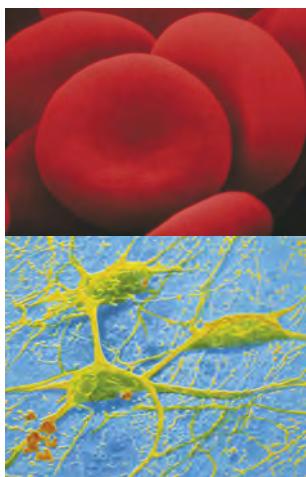


Figure 4: Red blood cells (top) and nerve cells (bottom) are both types of specialised cell.

Genes that are expressed get transcribed into mRNA, which is then translated into proteins. These proteins modify the cell — they determine the cell structure and control cell processes (including the expression of more genes, which produces more proteins).

Changes to the cell produced by these proteins cause the cell to become specialised. These changes are difficult to reverse, so once a cell has specialised it stays specialised. This is summarised in Figure 3.

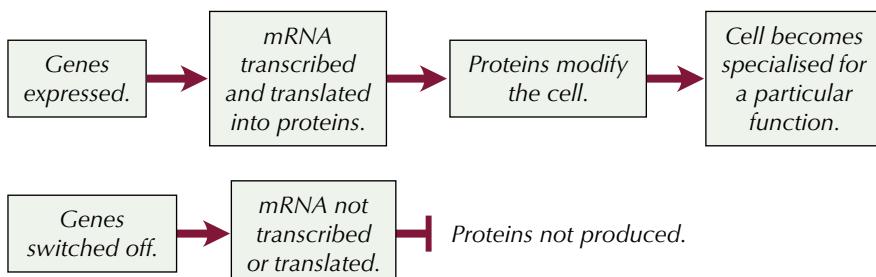


Figure 3: Summary of how cells become specialised through gene expression.

Example 1 — red blood cells

Red blood cells are produced from a type of stem cell in the bone marrow. They contain lots of haemoglobin and have no nucleus (to make room for more haemoglobin). The stem cell produces a new cell in which the genes for haemoglobin production are expressed. Other genes, such as those involved in removing the nucleus, are expressed too. Many other genes are not expressed (switched off), resulting in a specialised red blood cell.

Example 2 — nerve cells

Nerve cells have long axons and dendrites (branches), which connect them to other nerve cells. They're produced from stem cells in the neural tube. The stem cells produce new cells in which the genes that direct the axon to extend outwards are expressed. Genes that direct the dendrites to form are also expressed. Many other genes are switched off.

Cardiomyocytes

Cardiomyocytes (see Figure 5) are heart muscle cells that make up a lot of the tissue in our hearts. In mature mammals, it's thought that they can't divide to replicate themselves. This meant that for ages, everyone thought that we weren't able to regenerate our own heart cells at all. This is a major problem if the heart becomes damaged, e.g. by a heart attack, or the cells became worn out through age.

Recent research however, has suggested that our hearts do have some regenerative capability. Some scientists now think that old or damaged cardiomyocytes can be replaced by new cardiomyocytes derived from a small supply of unipotent stem cells in the heart.

Some researchers think that this process could be constantly occurring, but haven't yet agreed on how quickly it happens. Some believe that it's a really slow process and that it's possible that some cardiomyocytes are never replaced throughout a person's entire lifetime. Others think that it's occurring more quickly, so that every cardiomyocyte in the heart is replaced several times in a lifetime.

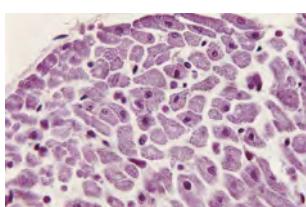


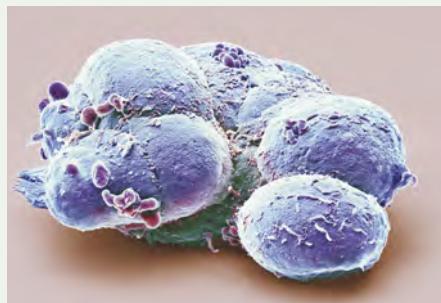
Figure 5: Light micrograph showing cardiomyocytes.

Practice Questions — Application

Q1 Spermatogonial stem cells are a type of stem cell found in the testes.
These cells can only differentiate into sperm cells.

What type of stem cell are spermatogonial stem cells?

Q2 The stem cells shown in the electron micrograph below can differentiate into any type of cell, except the cells that make up the placenta.



- a) What type of stem cells are they?
- b) Where would you find this type of stem cell?
- c) If these cells could also differentiate into placental cells what type of stem cells would they be?

Practice Questions — Fact Recall

Q1 What are stem cells?

Q2 Where are stem cells found in mammals?

Q3 Explain the difference between totipotent, multipotent and unipotent stem cells.

Q4 Describe how stem cells become specialised.

Q5 a) What is a cardiomyocyte?

b) What type of stem cell do some scientists think that new cardiomyocytes can be derived from?

Tip: The different types of stem cell have similar names so they're easy to get confused — make sure you take the time to learn which one is which.

Learning Objectives:

- Understand that pluripotent stem cells can divide in unlimited numbers and can be used in treating human disorders.
- Know that induced pluripotent stem cells (iPS cells) can be produced from adult cells using appropriate protein transcription factors.
- Be able to evaluate the use of stem cells in treating human disorders.

Specification Reference 3.8.2.1

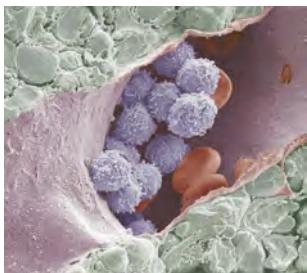


Figure 1: Bone marrow with developing white blood cells (blue).

Tip: These treatments aren't available yet, although some of them are at the clinical trial stage of testing.

6. Stem Cells in Medicine

Stem cells can be used in medicine to treat or cure various diseases. Some stem cell therapies are already being used, others are still being developed.

Stem cell therapies in existence

Since stem cells can divide into a range of specialised cell types, doctors and scientists think they could be used to replace cells damaged by illness or injury. Some stem cell therapies already exist for some diseases affecting the blood and immune system.

Bone marrow transplants

Bone marrow contains stem cells that can become specialised to form any type of blood cell. Bone marrow transplants can be used to replace the faulty bone marrow in patients that produce abnormal blood cells. The stem cells in the transplanted bone marrow divide and specialise to produce healthy blood cells.

This technique has been used successfully to treat leukaemia (a cancer of the blood or bone marrow) and lymphoma (a cancer of the lymphatic system). It has also been used to treat some genetic disorders, such as sickle-cell anaemia and severe combined immunodeficiency (SCID).

Example — SCID

Severe combined immunodeficiency (SCID) is a genetic disorder that affects the immune system. People with SCID have a poorly functioning immune system as their white blood cells (made in the bone marrow from stem cells) are defective. This means they can't defend the body against infections by identifying and destroying microorganisms. So SCID sufferers are extremely susceptible to infections.

Treatment with a bone marrow transplant replaces the faulty bone marrow with donor bone marrow that contains stem cells without the faulty genes that cause SCID. These then differentiate to produce functional white blood cells. These cells can identify and destroy invading pathogens, so the immune system functions properly.

Stem cell therapies of the future

As stem cells can divide into specialised cell types, scientists think they could be used to replace damaged tissues in a range of diseases. Scientists are researching the use of stem cells as treatment for lots of conditions, including:

- Spinal cord injuries — stem cells could be used to replace damaged nerve tissue.
- Heart disease and damage caused by heart attacks — stem cells could be used to replace damaged heart tissue.
- Bladder conditions — stem cells could be used to grow whole bladders, which are then implanted in patients to replace diseased ones.
- Respiratory diseases — donated windpipes can be stripped down to their simple collagen structure and then covered with tissue generated by stem cells. This can then be transplanted into patients.
- Organ transplants — organs could be grown from stem cells to provide new organs for people on donor waiting lists.

Sources of stem cells

To use stem cells scientists have to get them from somewhere.

There are three main potential sources of human stem cells:

1. Adult stem cells

These are obtained from the body tissues of an adult. For example, adult stem cells are found in bone marrow. They can be obtained in a relatively simple operation — with very little risk involved, but quite a lot of discomfort. Adult stem cells aren't as flexible as embryonic stem cells (see below) — they can only specialise into a limited range of cells, not all body cell types (they're multipotent). Although scientists are trying to find ways to make adult stem cells specialise into any cell type.

2. Embryonic stem cells

These are obtained from embryos at an early stage of development. Embryos are created in a laboratory using *in vitro* fertilisation (IVF) — egg cells are fertilised by sperm outside the womb. Once the embryos are approximately 4 to 5 days old, stem cells are removed from them and the rest of the embryo is destroyed. Embryonic stem cells can divide an unlimited number of times and develop into all types of body cells (they're pluripotent).

Tip: Embryos in the normal sense are made when a sperm fertilises an egg. But it is possible to create an embryo by artificially stimulating an unfertilised egg cell to divide.

3. Induced pluripotent stem cells (iPS cells)

iPS cells are created by scientists in the lab. The process involves 'reprogramming' specialised adult body cells so that they become pluripotent. The adult cells are made to express a series of **transcription factors** that are normally associated with pluripotent stem cells. The transcription factors cause the adult body cells to express genes that are associated with pluripotency.

Tip: Transcription factors are proteins that control whether or not genes are transcribed — see page 461 for more.

One of the ways that these transcription factors can be introduced to the adult cells is by infecting them with a specially-modified virus. The virus has the genes coding for the transcription factors within its DNA. When the virus infects the adult cell, these genes are passed into the adult cell's DNA, meaning that the cell is able to produce the transcription factors.

Induced pluripotent stem cells could become really useful in research and medicine in the future — see next page. At the moment though, more research into how similar they actually are to true pluripotent embryonic stem cells is needed before they can be properly utilised.

Ethical considerations

Obtaining stem cells from embryos created by IVF raises ethical issues because the procedure results in the destruction of an embryo that could become a fetus if placed in a womb. Some people believe that at the moment of fertilisation an individual is formed that has the right to life — so they believe that it's wrong to destroy embryos.

Some people have fewer objections to stem cells being obtained from egg cells that haven't been fertilised by sperm, but have been artificially activated to start dividing. This is because the cells couldn't survive past a few days and wouldn't produce a fetus if placed in a womb.

Some people think that scientists should only use adult stem cells because their production doesn't destroy an embryo. But adult stem cells can't develop into all the specialised cell types that embryonic stem cells can.

This is where induced pluripotent stem cells could prove really useful. They have the potential to be as flexible as embryonic stem cells, but, as



Figure 2: A pre-implantation IVF embryo.

Exam Tip

You might have to evaluate the use of stem cells in treating human disorders in the exam. Make sure you consider both sides of the argument and balance the benefits against the possible risks.

Tip: Adult stem cells taken from the patient's own tissues are also less likely to be rejected by the patient's body as they won't be seen as foreign.

they're obtained from adult tissue, there aren't the same ethical issues surrounding their use. It's also possible that iPS cells could be made from a patient's own cells. These iPS cells, which would be genetically identical to the patient's cells, could then be used to grow some new tissue or an organ that the patient's body wouldn't reject (rejection of transplants occurs quite often and is caused by the patient's immune system recognising the tissue as foreign and attacking it).

The decision makers in society have to take into account everyone's views when making decisions about important scientific work like stem cell research and its use to treat human disorders.

Benefits of stem cell therapy

People who make decisions about the use of stem cells to treat human disorders have to consider the potential benefits of stem cell therapies:

- They could save many lives — e.g. many people waiting for organ transplants die before a donor organ becomes available. Stem cells could be used to grow organs for those people awaiting transplants.
- It might even be possible to make stem cells genetically identical to a patient's own cells. These could then be used to grow some new tissue or an organ that the patient's body wouldn't reject.
- They could improve the quality of life for many people — e.g. they could be used to replace damaged cells in the eyes of people who are blind.

Practice Questions — Application

Q1 Sickle cell anaemia is an inherited disorder caused by a mutation in the haemoglobin protein. The mutated protein causes red blood cells to 'sickle' (twist into a crescent shape). The sickled cells then clump together, blocking capillaries and restricting blood flow.

All blood cells are produced from multipotent cells in the bone marrow. Describe how a bone marrow transplant could be used to cure sickle cell anaemia.

Q2 The cornea is the front part of the eye. Together with the lens, it refracts light into the eye and enables us to see. Scientists have been able to restore loss of vision caused by damage to the cornea using stem cells taken from healthy corneas.

- Suggest why stem cells are able to restore loss of vision in these circumstances.
- Patients with loss of vision in one eye are treated with stem cells taken from their other, healthy eye. Suggest two benefits of this.

Q3 Plasmids are small loops of DNA that can be inserted into cells. Suggest how plasmids could be used to produce induced pluripotent stem cells (iPS cells).

Q4 A team of scientists is investigating the use of embryonic stem cells in spinal cord injuries. Spinal cord injuries can cause paralysis, and patients may require long-term medical care and can have a lower quality of life. The team inject patients with embryonic stem cells taken from donated embryos left over from fertility treatment that would otherwise be discarded. If this injection works it could potentially allow for more movement in the patients. There are also other treatments being developed that use iPS cells.

Discuss the use of embryonic stem cells in this investigation.

Exam Tip

If you're asked to discuss the use of stem cells in the exam, you need to make your answer specific to the question asked to get full marks — don't just give general advantages and disadvantages.

7. Regulation of Transcription and Translation

Every cell in an organism contains the same DNA, but not all the proteins it codes for are made. This is because transcription and translation are controlled.

Controlling transcription

You should remember from Topic 4 that transcription is when a gene is copied from DNA into messenger RNA (mRNA). The enzyme responsible for synthesising mRNA from DNA is called RNA polymerase.

All the cells in an organism carry the same genes (DNA) but the structure and function of different cells varies. This is because not all the genes in a cell are expressed (transcribed and used to make a protein). Because different genes are expressed, different proteins are made and these proteins modify the cell — they determine the cell structure and control cell processes (including the expression of more genes, which produce more proteins). The transcription of genes is controlled by protein molecules called **transcription factors**.

The role of transcription factors

In eukaryotes, transcription factors move from the cytoplasm to the nucleus. In the nucleus they bind to specific DNA sites called **promoters**, which are found near the start of their target genes — the genes they control the expression of. Transcription factors control expression by controlling the rate of transcription.

Some transcription factors, called **activators**, stimulate or increase the rate of transcription — e.g. they help RNA polymerase bind to the start of the target gene and activate transcription. Other transcription factors, called **repressors**, inhibit or decrease the rate of transcription — e.g. they bind to the start of the target gene, preventing RNA polymerase from binding, stopping transcription. Figure 1 shows activators and repressors at work.

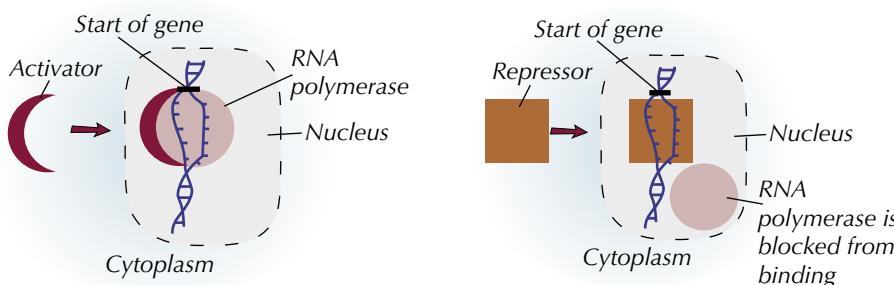


Figure 1: An activator (left) and a repressor (right) can control the rate of transcription by affecting RNA polymerase.

Oestrogen

The expression of genes can also be affected by other molecules in the cell, e.g. oestrogen. Oestrogen is a steroid hormone that can affect transcription by binding to a transcription factor called an oestrogen receptor, forming an oestrogen-oestrogen receptor complex (see Figure 2 on the next page).

Learning Objectives:

- Know that the transcription of target genes in eukaryotes can be stimulated or inhibited when specific transcriptional factors move from the cytoplasm into the nucleus.
- Understand the role of the steroid hormone, oestrogen, in initiating transcription.
- Know that in eukaryotes and some prokaryotes, translation of the mRNA produced from target genes can be inhibited by RNA interference (RNAi).
- Be able to interpret data from investigations into gene expression.

Specification Reference 3.8.2.2

Tip: Not all cell types have oestrogen receptors — so not all cells are affected by oestrogen.

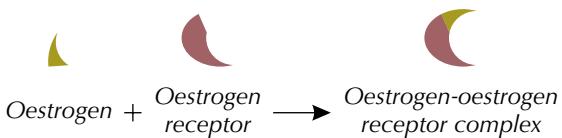


Figure 2: The formation of an oestrogen-oestrogen receptor complex.

The complex moves from the cytoplasm into the nucleus where it binds to specific DNA sites near the start of the target gene. The complex can act as an activator of transcription, e.g. helping RNA polymerase bind to the start of the target gene (see Figure 3).

Tip: In some cells, the oestrogen-oestrogen receptor complex can act as a repressor of transcription instead of an activator. It depends on the type of cell and the target gene.

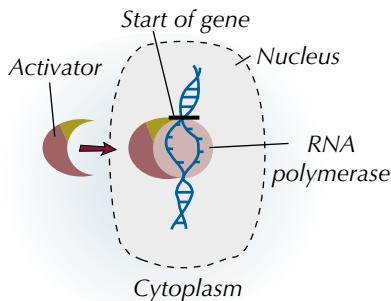


Figure 3: An oestrogen-oestrogen receptor complex activating transcription.

RNAi

Tip: RNAi molecules are small lengths of non-coding RNA (they don't code for proteins).

Tip: Unlike mRNA and tRNA, siRNA is double-stranded.

Tip: Double-stranded siRNA is unwound into two single-stranded siRNA molecules by an enzyme.

In eukaryotes, gene expression is also affected by RNA interference (RNAi). RNAi is where small, double-stranded RNA molecules stop mRNA from target genes being translated into proteins. A similar process to RNAi can also occur in prokaryotes. The molecules involved in RNAi are called siRNA (small interfering RNA) and miRNA (microRNA). Here's how RNAi works:

siRNA (and miRNA in plants)

Once mRNA has been transcribed, it leaves the nucleus for the cytoplasm (see Figure 4).

In the cytoplasm, double-stranded siRNA associates with several proteins and unwinds. One of the resulting single strands of siRNA is selected and the other strand is degraded (broken down) — see Figure 5.

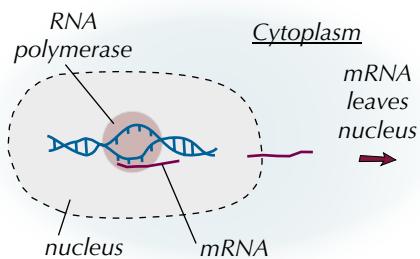


Figure 4: mRNA leaves the nucleus after transcription.

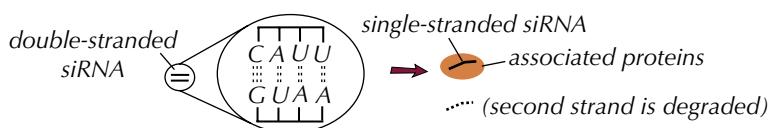


Figure 5: siRNA associates with proteins in the cytoplasm.

Tip: siRNA is actually about 20-25 nucleotides long (this diagram just shows a short section).

The single strand of siRNA then binds to the target mRNA. The base sequence of the siRNA is complementary to the base sequence in sections of the target mRNA (see Figure 6).

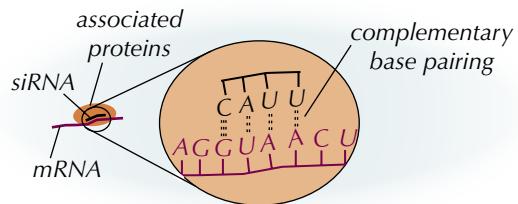


Figure 6: siRNA binds to the target mRNA.

The proteins associated with the siRNA cut the mRNA into fragments — so it can no longer be translated. The fragments then move into a processing body, which contains ‘tools’ to degrade them (see Figure 7).

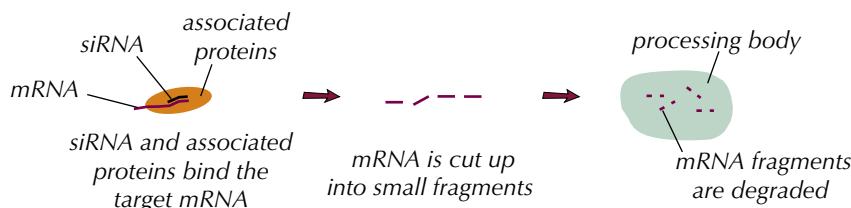


Figure 7: siRNA affects gene expression by directing the target mRNA to be cut up.

A similar process happens with miRNA in plants. Like siRNA, the base sequence of plant miRNA is complementary to its target mRNA sequence and so binding results in the cutting up and degradation of the mRNA. However, its production in the cell is similar to that of mammalian miRNA — see below.

Tip: siRNA has a potential use in treating genetic disorders, for example stopping a known harmful gene from being expressed. siRNA molecules with a base sequence complementary to the mRNA from that gene could be inserted into the affected cells — they will bind to the mRNA and so block translation of that protein.

miRNA in mammals

In mammals, the miRNA isn't usually fully complementary to the target mRNA. This makes it less specific than siRNA and so it may target more than one mRNA molecule.

When miRNA is first transcribed, it exists as a long, folded strand. It is processed into a double stand, and then into two single strands, by enzymes in the cytoplasm.

Like siRNA, one strand associates with proteins and binds to target mRNA in the cytoplasm. Instead of the proteins associated with miRNA cutting mRNA into fragments, the miRNA-protein complex physically blocks the translation of the target mRNA. The mRNA is then moved into a processing body, where it can either be stored or degraded. When it's stored, it can be returned and translated at another time.

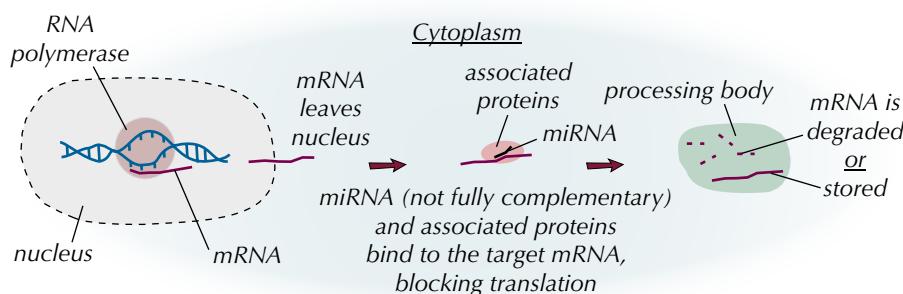
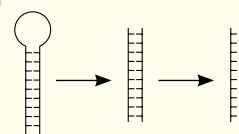


Figure 8: miRNA affects gene expression by blocking the translation of mRNA.

Tip: miRNA is processed like this:



The unused single strand is degraded in the cytoplasm (like the second strand of siRNA, see previous page).

Practice Questions — Application

- Q1 Rett syndrome is a neuro-developmental disorder caused by a mutation in the MECP2 gene. The protein produced by the gene is a transcription factor which acts as a repressor and is needed for normal functioning of nerve cells. Mutations in the gene often result in reduced production of the protein. Suggest how a mutation in the MECP2 gene causes Rett syndrome.
- Q2 AMD is a medical condition which results in loss of vision because of damage to the retina. It is caused by the expression of multiple genes and by environmental factors. A treatment is being developed using siRNA. Suggest how siRNA could be used to treat AMD.

Interpreting data on gene expression

Exam Tip

You don't need to learn all the information in this example for your exam, but do make sure you understand what the results of the experiment tell you about how the expression of the gene is controlled.

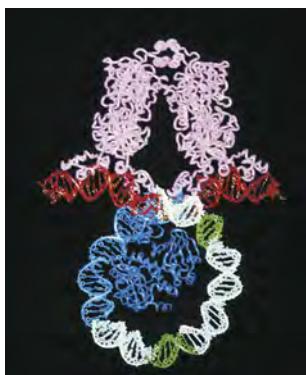


Figure 10: Molecular model of the lac repressor (pink) binding to DNA.

Exam Tip

The data you are given in the exam won't necessarily be a table. You could be given a bar chart, scatter graph, or even a frequency table. Make sure you're comfortable analysing data in all its forms.

You could get a question in the exam where you have to interpret data about gene expression. It could be on anything you've learnt on pages 461-463 (e.g. transcription factors, oestrogen or RNAi) or it could be on epigenetic control of gene expression (see pages 466-468). Below is an example of a gene expression system in bacteria and an experiment that investigates how it works.

Example

The lac repressor

E. coli is a bacterium that respires glucose, but it can use lactose if glucose isn't available. If lactose is present, *E. coli* makes an enzyme (β -galactosidase) to digest it. But if there's no lactose, it doesn't waste energy making an enzyme it doesn't need.

The enzyme's gene is only expressed when lactose is present. The production of the enzyme is controlled by a transcription factor — the *lac* repressor. When there's no lactose, the *lac* repressor binds to the DNA at the start of the gene, stopping transcription. When lactose is present it binds to the *lac* repressor, stopping it binding to the DNA, so the gene is transcribed (see Figure 9).



Figure 9: Lactose can activate transcription by stopping the lac repressor from binding to the DNA, so that RNA polymerase can bind instead.

The experiment

Different *E. coli* mutants were isolated and grown in different media, e.g. with lactose or glucose. The mutants have mutations (changes in their DNA bases, see page 443) that mean they act differently from normal *E. coli*, e.g. they produce β -galactosidase when grown with glucose.

To detect whether active (working) β -galactosidase was produced, a chemical that turns yellow in the presence of active β -galactosidase was added to the medium. The production of mRNA that codes for β -galactosidase was also measured. The results are shown in the table.

| Medium | Mutant | mRNA | Colour |
|---------|----------|------|-----------|
| Glucose | Normal | No | No yellow |
| Lactose | Normal | Yes | Yellow |
| Glucose | Mutant 1 | Yes | Yellow |
| Lactose | Mutant 1 | Yes | Yellow |
| Glucose | Mutant 2 | No | No yellow |
| Lactose | Mutant 2 | Yes | No yellow |

In mutant 1, mRNA and active β -galactosidase were produced even when they were grown with only glucose — the gene is always being expressed. This suggests that mutant 1 has a faulty *lac* repressor, e.g. in the absence of lactose the repressor isn't able to bind DNA, so transcription can occur and mRNA and active β -galactosidase are produced.

In mutant 2, mRNA is produced but active β -galactosidase isn't when lactose is present — the gene is being transcribed but it isn't producing active β -galactosidase. This suggests mutant 2 is producing faulty β -galactosidase, e.g. because a mutation has affected its active site.

Practice Questions — Application

The production of tryptophan in bacteria is controlled by a repressor. When tryptophan is present in the bacterial cell it binds to the repressor, allowing it to bind to promoters near its target genes.

A team of scientists have studied the activity of normal bacteria and bacteria that have a mutation in the tryptophan repressor gene, by measuring the amount of tryptophan mRNA present in the bacteria. Their results are shown in the table below.

| Starter culture | Target mRNA (arbitrary units) |
|--|----------------------------------|
| Normal bacteria in the presence of tryptophan. | 0.13 |
| Normal bacteria without tryptophan present. | 9.30 |
| Mutant bacteria in the presence of tryptophan. | 9.28 |
| Mutant bacteria without tryptophan present. | 9.33 |

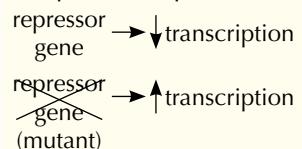
- Q1 Suggest three factors that should be controlled in this experiment.
- Q2 In normal bacteria, the presence of tryptophan prevents the production of more tryptophan.
- Use evidence from the table to explain how the presence of tryptophan prevents the production of more tryptophan in normal bacteria.
 - Suggest why it is beneficial for bacteria to be able to control their tryptophan production.
- Q3 Describe the results for the mutant bacteria, and suggest an explanation.

Exam Tip

Tryptophan is just a type of amino acid — but you don't actually need to know this to answer the question. If something unfamiliar like this comes up in the exam, don't let it throw you. Just apply what you do know to the information you're given and you should be able to get all the marks.

Tip: You don't need to know the exact details of an experiment to be able to work out the kind of controls it should include.

Tip: Questions like this on repressors can start to become a bit confusing if you're not careful... so take your time and work through the questions carefully. A quick sketch might help, for example:



Practice Questions — Fact Recall

- Q1 What are transcription factors?
- Q2 What is:
- an activator?
 - a repressor?
- Q3 Explain why not all cells are affected by oestrogen.
- Q4 Describe the process by which oestrogen activates the transcription of a gene.
- Q5 What is RNAi and how does it work? (Use siRNA as an example.)
- Q6 Why might a mammalian miRNA molecule target a greater range of mRNA molecules than siRNA?

Learning Objectives:

- Recall the process of epigenetic control of gene expression in eukaryotes.
- Know that epigenetics involves heritable changes in gene function, without changes to the base sequence of DNA.
- Understand that epigenetic changes are caused by changes in the environment that inhibit transcription by:
 - increased methylation of the DNA or
 - decreased acetylation of associated histones.
- Understand the relevance of epigenetics on the development and treatment of disease, especially cancer.

Specification Reference 3.8.2.2

Tip: Epigenetic marks are important for cell specialisation. Most epigenetic marks are removed between generations because cells from the fertilised egg need to be able to become any type of cell (i.e. they need to be totipotent — see p. 455).

Tip: A methyl group is a $-CH_3$ group.

Tip: The methyl group is attached to cytosine by enzymes called DNA methyltransferases.

8. Epigenetic Control of Gene Expression

Gene expression isn't just controlled by transcription factors. Epigenetic changes also play a part in whether a gene is expressed or not.

How does epigenetic control work?

In eukaryotes, epigenetic control can determine whether a gene is switched on or off — i.e. whether the gene is expressed (transcribed and translated) or not. It works through the attachment or removal of chemical groups (known as epigenetic marks) to or from DNA or histone proteins (see below and next page). These epigenetic marks don't alter the base sequence of DNA. Instead, they alter how easy it is for the enzymes and other proteins needed for transcription to interact with and transcribe the DNA.

Epigenetic changes to gene expression play a role in lots of normal cellular processes and can also occur in response to changes in the environment — e.g. pollution and availability of food.

Inheriting epigenetic changes

Organisms inherit their DNA base sequence from their parents. Most epigenetic marks on the DNA are removed between generations, but some escape the removal process and are passed on to offspring. This means that the expression of some genes in the offspring can be affected by environmental changes that affected their parents or grandparents.

Example

Epigenetic changes in some plants in response to drought have been shown to be passed on to later generations.

Controlling gene expression

There are several epigenetic mechanisms used to control gene expression. You need to know about methylation of DNA and the acetylation of histones.

Increased methylation of DNA

Methylation is when a methyl group (an example of an epigenetic mark) is attached to the DNA coding for a gene.

The group always attaches at a CpG site, which is where a cytosine and guanine base are next to each other in the DNA (linked by a phosphodiester bond). Increased methylation changes the DNA structure so that the transcriptional machinery (enzymes, etc.) can't interact with the gene — so the gene is not expressed (i.e. it's switched off).

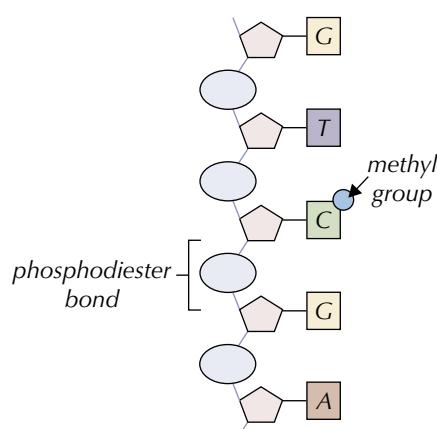


Figure 1: DNA strand with a methyl group attached.

Decreased acetylation of histones

Histones are proteins that DNA wraps around to form chromatin, which makes up chromosomes. Chromatin can be highly condensed or less condensed. How condensed it is affects the accessibility of the DNA and whether or not it can be transcribed.

Histones can be epigenetically modified by the addition or removal of **acetyl groups** (which are another example of an epigenetic mark). When histones are acetylated, the chromatin is less condensed. This means that the transcriptional machinery can access the DNA, allowing genes to be transcribed. When acetyl groups are removed from the histones, the chromatin becomes highly condensed and genes in the DNA can't be transcribed because the transcriptional machinery can't physically access them. Histone deacetylase (HDAC) enzymes are responsible for removing the acetyl groups.

Tip: An acetyl group is a -COCH_3 group.

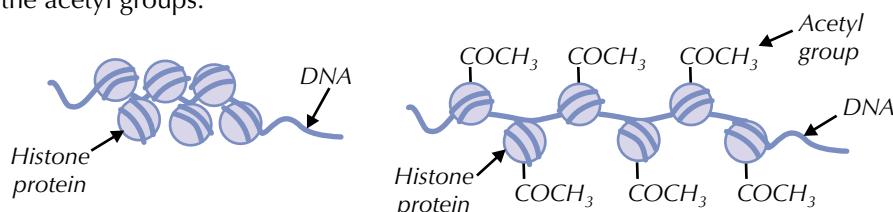


Figure 2: Highly condensed chromatin (left) and less condensed chromatin (right).

Development of disease

You've already seen on page 449 how epigenetics can play a role in the development of disease, with the fact that abnormal methylation of tumour suppressor genes and oncogenes can cause cancer. However, the role of epigenetics in disease doesn't stop there. It can play a role in the development of many other diseases, including Fragile-X syndrome, Angelman syndrome and Prader-Willi syndrome.

Example — Fragile-X syndrome

Fragile-X syndrome is a genetic disorder that can cause symptoms such as learning and behavioural difficulties, as well as characteristic physical features. It's caused by a heritable duplication mutation (see page 443) in a gene on the X chromosome, called FMR1. The mutation results in the short DNA sequence CGG being repeated many more times than usual.

These repeats mean that there are lots more CpG sites (see previous page) in the gene than usual. More CpG sites result in increased methylation of the gene, which switches it off. Because the gene is switched off, the protein that it codes for isn't produced. It's the lack of this protein that causes the symptoms of the disease.

Tip: The FMR1 CGG sequence is normally repeated between 5 and 40 times, but in the mutated gene it's repeated over 200 times.

Example — Angelman syndrome

Angelman syndrome is a genetic disorder that affects the nervous system and causes symptoms such as delayed development and motor problems. It's caused by a mutation or deletion of a region of chromosome 15. In most cases, the maternal allele in the affected region of chromosome 15 is missing. The paternal allele is present in the cell, but it is switched off by methylation and so the gene is not transcribed. Like for fragile-X syndrome this means that a protein is not produced, which leads to the symptoms of the disorder.

Exam Tip

You don't need to remember these specific examples for your exam, but you do need to understand how epigenetics can cause disorders.

Tip: Epigenetic changes are a lot easier to treat than DNA sequence mutations (due to their reversibility).

Tip: There's more about the function of tumour suppressor genes on page 447.

Tip: If these drugs that counteract epigenetic changes also activate transcription in normal cells, the cells could become cancerous, creating the very problem that the drugs are supposed to be treating.

Example — Prader-Willi syndrome

Prader-Willi syndrome is a genetic disorder characterised by developmental issues and excessive hunger. Most cases of Prader-Willi syndrome are caused by the loss of function of genes from the same region of chromosome 15 as Angelman syndrome. However, in this case it is the paternal allele that's usually transcribed and so the syndrome results when the deletion occurs on the paternal chromosome. The maternal gene is silenced by methylation and so is unable to compensate. The lack of the protein encoded by this gene leads to the disorder.

Treating disease

Epigenetic changes are reversible, which makes them good targets for new drugs to combat diseases they cause. These drugs are designed to counteract the epigenetic changes that cause the diseases.

For example, increased methylation is an epigenetic change that can lead to a gene being switched off. Drugs that stop DNA methylation can sometimes be used to treat diseases caused in this way.

Example

The drug azacitidine is used in chemotherapy for types of cancer that are caused by increased methylation of tumour suppressor genes. Tumour suppressor genes usually slow cell division, so if they are switched off by methylation, cells are able to divide uncontrollably and can form a tumour. Azacitidine inhibits the methylation of these genes by physically blocking the enzymes involved in the process.

Decreased acetylation of histones can also lead to genes being switched off. HDAC inhibitor drugs, e.g. romidepsin, can be used to treat diseases that are caused in this way — including some types of cancer. These drugs work by inhibiting the activity of histone deacetylase (HDAC) enzymes, which are responsible for removing the acetyl groups from the histones. Without the activity of HDAC enzymes, the genes remain acetylated and the proteins they code for can be transcribed.

The problem with developing drugs to counteract epigenetic changes is that these changes take place normally in a lot of cells, so it's important to make sure the drugs are as specific as possible. E.g. drugs used in cancer therapies can be designed to only target dividing cells to avoid damaging normal body cells.

Practice Questions — Application

A single genotype of plant was subjected to nutrient deprivation and was left to produce a second generation of plants. Most plants of the second generation were found to have an increased number of methylated genes.

Q1 What would be the effect of methylation on the affected genes?

Q2 Suggest why a single genotype of parental plant was used.

Q3 A small number of individuals in the second generation did not show the methylation changes. Explain why.

Practice Questions — Fact Recall

Q1 Give two examples of epigenetic marks.

Q2 How can histone acetylation affect gene expression?

9. Evaluating Data on Phenotypes

Both genetic and environmental factors influence the phenotype of an organism. You need to be able to evaluate data on their relative influences — this may just pop up in your exam...

Learning Objective:

- Be able to evaluate appropriate data for the relative influences of genetic and environmental factors on phenotype.

Specification Reference 3.8.2.2

Evaluating data about influences on phenotypes

The **phenotype** (characteristics) of an organism is the result of the organism's **genotype** and the interaction of its genotype with the environment (see page 378). It's not always clear how much a phenotype is influenced by genes and how much it's influenced by the environment — have a look at these two examples:

Example 1 — Overeating

Overeating was thought to be caused only by environmental factors, like an increased availability of food in developed countries. It was later discovered that food consumption increases brain dopamine levels in animals. Once enough dopamine was released, people would stop eating.

Researchers discovered that people with one particular allele had 30% fewer dopamine receptors. They found that people with this particular allele were more likely to overeat — they wouldn't stop eating when dopamine levels increased. Based on this evidence, scientists now think that overeating has both genetic and environmental causes.

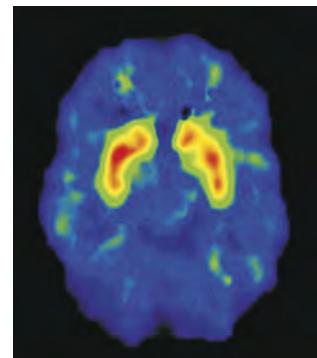


Figure 1: A coloured PET scan showing a slice through the brain. Brighter areas show high dopamine activity.

Example 2 — Antioxidants

Many foods in our diet contain antioxidants — compounds that are thought to play a role in preventing chronic diseases. Foods such as berries contain high levels of antioxidants. Scientists thought that the berries produced by different species of plant contained different levels of antioxidants because of genetic factors.

But experiments that were carried out to see if environmental conditions affected antioxidant levels found that environmental conditions caused a great deal of variation. Scientists now believe that antioxidant levels in berries are due to both genetic and environmental factors.

Twin studies

In the exam, you might have to evaluate data on the relative influences of genes and the environment on phenotype. This data may come from twin studies.

Studies of identical twins are extremely useful when trying to determine what's due to environmental factors and what's due to genetic factors. These twins are genetically identical, so any differences in phenotype must be entirely due to environmental factors. If a characteristic is very similar in identical twins, genetics probably plays a more important role. But if a characteristic is different between the twins, the environment must have a larger influence.

Tip: Identical twins have very similar epigenetic marks when they are born and in the first years of their life. Different epigenetic changes occur in each twin as they get older. Environmental factors that can affect the epigenome (the epigenetic marks that have been added to the entire genome) include diet, physical exercise and stress.

Example

Studies of identical twins can be useful for determining the importance of genetic and environmental factors in the development of certain diseases. Comparisons between the prevalence of Alzheimer's disease in identical twins and non-identical twins have shown that the disease has a genetic risk. However, the disease is not always found in both identical twins, which suggests that environmental factors also play a part. In fact, it's thought that genetics account for about 80% of the risk.

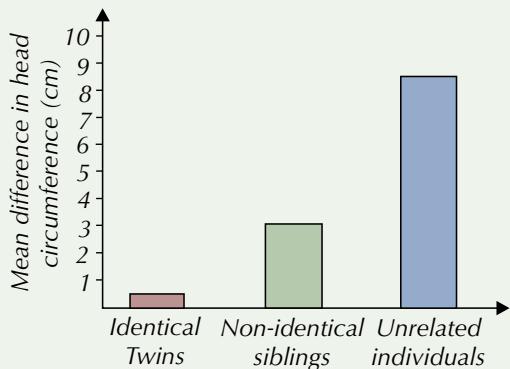
Data that comes from twin studies involving a large sample size (i.e. lots of pairs of twins) is better for drawing valid conclusions than data based on a small sample size. That's because a large sample size is more representative of the population.

Practice Question — Application

- Q1 A twin study was performed to determine whether head circumference is influenced mainly by environmental factors or by genetic factors.

25 pairs of identical twins were selected for the study and the mean difference in the head circumference of each pair was calculated. The same was done for 25 pairs of non-identical siblings and 25 pairs of unrelated individuals. The results are shown on the right.

- Describe the data.
- Do you think that genetic or environmental factors have a larger effect on head circumference? Explain your answer.

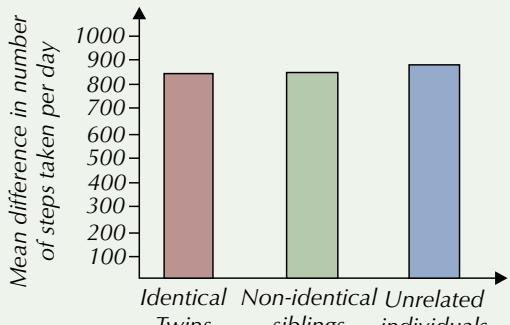


Tip: It's not always clear what the main cause of differences in phenotype is, so you need to be careful when drawing any conclusions about these differences.

A similar study was performed on adults to determine the effects of genetic and environmental factors on activity levels. Pairs of identical twins, pairs of non-identical siblings and pairs of unrelated individuals were asked

to wear a pedometer and the mean difference in steps taken per day was recorded. The results are shown on the right.

- Explain what the results show about the role of genetics in determining activity levels.



Practice Question — Fact Recall

- Q1 Why are studies using identical twins useful for determining the effect of both environmental and genetic factors on a phenotype?

Section Summary

Make sure you know...

- That mutations are changes to the base sequence of DNA that can occur during DNA replication.
- That mutations can include substitution, deletion, addition, duplication, inversion and translocation of bases.
- That some mutations can affect polypeptide function by altering the amino acid sequence.
- That some mutations only change one triplet code and not all of these mutations change the amino acid because the genetic code is degenerate.
- That some mutations result in a frameshift (all base triplets downstream of the mutation are changed).
- How the type of mutation relates to its effect on the encoded polypeptide.
- That mutations can occur spontaneously, e.g. if mistakes are made during DNA replication, and the rate of mutations is increased by mutagenic agents (e.g. UV radiation, ionising radiation).
- That tumour suppressor genes slow down cell division and proto-oncogenes stimulate cell division.
- That mutated tumour suppressor genes can be inactivated and fail to stop cell division. This allows cells to divide uncontrollably, leading to the formation of a tumour.
- That mutated proto-oncogenes (called oncogenes) stimulate cells to divide uncontrollably leading to the formation of a tumour.
- That malignant tumours are cancerous, grow rapidly and invade (and destroy) surrounding tissues, while benign tumours are not cancerous, grow slowly and are covered in fibrous tissue.
- That abnormal methylation of tumour suppressor genes and proto-oncogenes leads to changes in those cells that promote the development of tumours.
- That increased exposure to oestrogen over a long period of time is thought to increase the risk of breast cancer and the theories behind why this is.
- How to evaluate evidence of correlations between genetic and environmental factors, and cancer.
- How to interpret information about proto-oncogenes and tumour suppressor genes in relation to the prevention, treatment and cure of cancers.
- That totipotent stem cells can mature (develop) into any type of body cell and that they occur only for the first few cell divisions in mammalian embryos, and that pluripotent stem cells occur in mammalian embryos and that they can mature into any type of body cell (except placental cells).
- That multipotent and unipotent stem cells are found in mature mammals and can only form a limited number of cell types.
- That stem cells become specialised during their development by only translating some of their DNA.
- That cardiomyocytes (heart muscle cells) can be produced by unipotent stem cells in the heart.
- That pluripotent stem cells can be used for treating human disorders because they can divide an unlimited number of times and divide into a range of specialised cell types.
- How induced pluripotent stem cells can be produced from adult cells by using transcription factors.
- That there are many benefits to stem cell therapy, but that there are also ethical issues to consider.
- How transcription and translation are regulated by transcription factors.
- That oestrogen can affect translation by binding to a transcription factor.
- How translation is regulated by RNA interference (RNAi).
- How to interpret data from investigations into gene expression.
- That the process of epigenetic control in eukaryotes involves epigenetic changes that determine whether a gene is switched on or off.
- That epigenetic changes are heritable changes in gene function that don't change the DNA sequence.
- That epigenetic changes are caused by changes in the environment, leading to increased methylation of DNA or decreased acetylation of histones.
- How epigenetic changes can cause disease and how these diseases could be treated.
- How to evaluate data on the effect of environmental and genetic factors on phenotypes.

Exam-style Questions

- 1 A genus of bacteria that produce many important antibiotics is being investigated. One species produces a blue-coloured antibiotic. Production of the antibiotic is controlled by a transcription factor that binds to the start region of the antibiotic gene. **Figure 1** shows part of the DNA sequence for the transcription factor gene and the same part of the DNA sequence from a mutant strain of the bacteria.

Figure 1

| | | | | | | | | | | | | | | | | |
|-----------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal sequence | T | C | G | C | C | A | A | C | A | A | C | A | C | T | C | G |
| Mutant sequence | T | G | C | C | A | A | C | A | A | C | A | C | T | C | G | |

Table 1

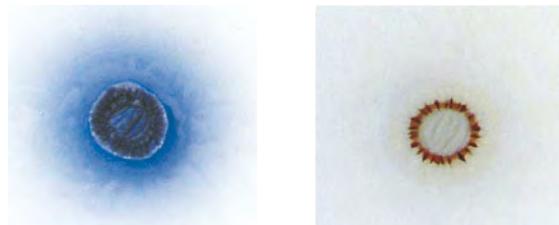
- 1.1 Name the type of mutation in the sequence above.
(1 mark)
- 1.2 Using the information provided in **Table 1**, give the mRNA and amino acid sequences for the mutant DNA sequence.
(2 marks)
- 1.3 Scientists investigated how the amounts of the transcription factor protein, antibiotic mRNA and the antibiotic itself were affected in the mutant. Their results are shown in **Table 2** and **Figure 2**.

| mRNA codon | Amino acid |
|------------|---------------|
| GAA | Glutamic acid |
| GUG | Valine |
| GUU | Valine |
| GCG | Alanine |
| AGC | Serine |
| ACG | Threonine |
| UCG | Serine |
| UUG | Leucine |

Table 2

| Bacteria | Transcription factor protein (arbitrary units) | Antibiotic mRNA (arbitrary units) |
|----------|---|--------------------------------------|
| Normal | 7.9 | 8.2 |
| Mutant | 7.7 | 0.9 |

Figure 2

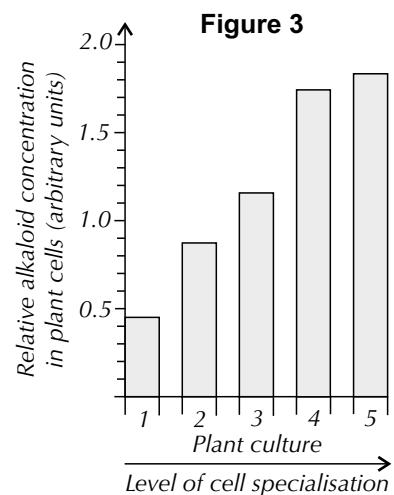


Antibiotic production in normal bacteria.
Antibiotic production in mutant bacteria.

Is the transcription factor an activator or a repressor?
Use the evidence provided above to explain your answer.

(4 marks)

- 2** A mutation in the APC gene is found in the majority of colon cancers. A smaller number have a mutation in the gene which codes for β -catenin. The APC protein helps to mark β -catenin for destruction in the cytoplasm. This prevents β -catenin from moving to the nucleus and activating the transcription of genes needed for cell division. Mutations in the APC gene prevent the protein produced from carrying out its function.
- 2.1** Of the two genes described above, which is a tumour suppressor gene and which is a proto-oncogene? Explain your answer. (2 marks)
- 2.2** Suggest how a mutation in each of these genes could lead to cancer. (2 marks)
- 2.3** Mutations that result in a non-functioning APC protein are usually caused by base deletions. Explain how a deletion of one or more bases could result in a non-functioning APC protein. (3 marks)
- 2.4** People with a hereditary mutation in the APC gene have a condition called FAP. FAP sufferers will almost certainly develop colon cancer by the age of forty if preventative measures are not taken. Suggest one way in which knowing you have a hereditary APC mutation could help in the prevention, diagnosis or treatment of colon cancer. (1 mark)
- 3** Plant stem cells are totipotent. Plant cells and tissues can be grown artificially from plant stem cells — this is called tissue culture. Alkaloids are useful chemicals produced by plants. They can be harvested from plants grown by tissue culture. An investigation was carried out into the best time to harvest alkaloids produced via tissue culture. Alkaloids were collected from five different cultures of the same plant species. The cells in each tissue culture were at a different stage of specialisation. The results are shown in **Figure 3**.
- 3.1** Describe the results shown in the graph. (1 mark)
- 3.2** Calculate the percentage change in alkaloid concentration between culture 1 and culture 5. (1 mark)
- 3.3** Auxins promote cell growth and division, but high concentrations of auxins can reduce cell specialisation. When plants are grown via tissue culture for alkaloid production, auxins are often added to the growth medium during the early stages, but removed later on. Suggest why this is the case. (2 marks)
- 3.4** Auxins can affect whether or not certain genes are expressed. Describe and explain how this could affect cell specialisation. (5 marks)



Learning Objectives:

- Know that sequencing projects have read the genomes of a wide range of organisms, including humans.
- Know that determining the genome of simpler organisms allows the sequences of the proteins that derive from the genetic code (the proteome) of the organism to be determined and that this may have many applications, including the identification of potential antigens for use in vaccine production.
- Know that in more complex organisms, the presence of non-coding DNA and of regulatory genes means that knowledge of the genome cannot easily be translated into the proteome.
- Know that sequencing methods are continuously updated and have become automated.

Specification Reference 3.8.3

Tip: Remember, vaccines contain antigens that cause your body to produce memory cells. If you're later infected by a pathogen with the same antigens, your memory cells will quickly recognise it and divide to produce antibodies against it.

1. Genome Projects

A genome is the entire set of DNA, including all the genes, in an organism. In genome projects, scientists work to determine the complete genome sequence of an organism. Their success depends on the complexity of the organism and the technology that is available.

Sequencing genomes

Improvements in technology have allowed us to sequence the genomes of a variety of organisms, from bacteria to humans. Gene sequencing methods only work on fragments of DNA, so if you want to sequence the entire genome of an organism, you need to chop it up into smaller pieces first. The smaller pieces are sequenced and then put back in order to give the sequence of the whole genome.

Example

The Human Genome Project, which was completed in 2003, mapped the entire sequence of the human genome for the first time. In 1990, scientists round the world joined together to attempt to sequence all 3 billion base pairs in the human genome. The aim was to improve our understanding of the genetic factors in human disease, so that new ways to diagnose and treat illness could be developed. Now that we have the complete sequence of the human genome, genes causing inherited diseases can be found in days rather than the years it took previously.

Sequencing proteomes

The proteome of an organism is all the proteins that are made by it. You might remember from Topic 4 that while some parts of the genome code for specific proteins, some parts don't code for anything at all (the DNA is non-coding).

Simple organisms

Simple organisms, such as bacteria, don't have much non-coding DNA. This means it is relatively easy to determine their proteome from the DNA sequence of their genome. This can be useful in medical research and development. For example, identifying the protein antigens on the surface of disease-causing bacteria and viruses can help in the development of vaccines to prevent the disease.

Example

N. meningitidis group B bacteria cause meningitis B. Sequencing the genome of these bacteria helped researchers identify antigens for use in developing a vaccine against the disease.

Being able to determine the proteomes of disease-causing bacteria and viruses also allows pathogens to be monitored during outbreaks of disease, which can lead to better management of the spread of infection, and can help to identify antibiotic resistance factors (e.g. mechanisms of antibiotic resistance).

Complex organisms

More complex organisms contain large sections of non-coding DNA. They also contain complex regulatory genes, which determine when the genes that code for particular proteins should be switched on and off. This makes it more difficult to translate their genome into their proteome, because it's hard to find the bits that code for proteins among the non-coding and regulatory DNA. However, work is being done on the human proteome. The codes for more than 30 000 human proteins have been identified so far.

Developing new sequencing methods

In the past, many sequencing methods were labour-intensive, expensive and could only be done on a small scale.

Example

During the 1970s, Frederick Sanger developed a technique in which a sample of DNA was tagged with radioactive bases, separated into four lanes on a gel and allowed to migrate. The result was photographed by X-ray. As each lane represented one of the four bases, the sequence of the DNA could be worked out by combining the results in each lane. This was a time-consuming process as only one sample could be run at a time and X-ray photographs had to be taken manually.

Tip: Sanger had to split the DNA sample into four and replicate each portion with a different radioactive base to produce the sample for each lane.

Now these techniques are often automated, more cost-effective and can be done on a large scale.

Example

Pyrosequencing is a recently developed technique that can sequence around 400 million bases in a ten hour period (which is super fast compared to older techniques).

With newer, faster techniques such as pyrosequencing available, scientists can now sequence whole genomes much more quickly.

Practice Questions — Fact Recall

- Q1 What is the main aim of a genome project?
- Q2 a) What is the proteome of an organism?
 - b) Give one example of why determining the proteome of an organism is useful for medical research and development.
- Q3 Why is it more difficult to determine the proteome of a more complex organism than a simple organism?
- Q4 In what ways have sequencing methods been improved since they were first introduced?



Figure 1: Automated DNA sequencer used for the Human Genome Project.

Learning Objectives:

- Know that recombinant DNA technology involves the transfer of fragments of DNA from one organism, or species, to another and that since the genetic code is universal, as are transcription and translation mechanisms, the transferred DNA can be translated within cells of the recipient (transgenic) organism.
- Know that fragments of DNA can be produced by conversion of mRNA to complementary DNA (cDNA), using reverse transcriptase.
- Know that fragments of DNA can be produced using restriction enzymes to cut a fragment containing the desired gene from DNA.
- Know that fragments of DNA can be produced by creating the gene in a 'gene machine'.

Specification Reference 3.8.4.1

Tip: Remember that DNA is copied into mRNA during transcription.

Tip: The cDNA is a complementary copy of the mRNA because of specific base pairing.

2. Making DNA Fragments

Recombinant DNA technology allows us to combine genetic material from different sources. The first step in recombinant DNA technology is often making a DNA fragment — a bit of DNA containing a gene.

Recombinant DNA technology

Recombinant DNA technology involves transferring a fragment of DNA from one organism to another. Because the genetic code is universal (the same DNA base triplets code for the same amino acids in all living things), and because transcription and translation mechanisms are pretty similar too, the transferred DNA can be used to produce a protein in the cells of the recipient organism. The recipient and donor organisms don't even have to be from the same species. This can be pretty useful — see page 485. Organisms that contain transferred DNA are known as transgenic organisms.

Methods for making DNA fragments

In order to transfer a gene from one organism to another, you first need to get a DNA fragment containing the gene you're interested in (the target gene). There are three ways that DNA fragments can be produced:

Method 1 — using reverse transcriptase

Most cells only contain two copies of each gene, making it difficult to obtain a DNA fragment containing the target gene. But cells that produce the protein coded for by the target gene will contain many mRNA molecules that are complementary to the gene — so mRNA is often easier to obtain. The mRNA molecules can be used as templates to make lots of DNA. The enzyme, **reverse transcriptase**, makes DNA from an RNA template. The DNA produced is called **complementary DNA** (cDNA).

Example

Pancreatic cells produce the protein insulin. They have loads of mRNA molecules complementary to the insulin gene, but only two copies of the gene itself. So reverse transcriptase could be used to make cDNA from the insulin mRNA.

To make cDNA, mRNA is first isolated from cells. Then it's mixed with free DNA nucleotides and reverse transcriptase. The reverse transcriptase uses the mRNA as a template to synthesise new strands of cDNA — see Figure 1.

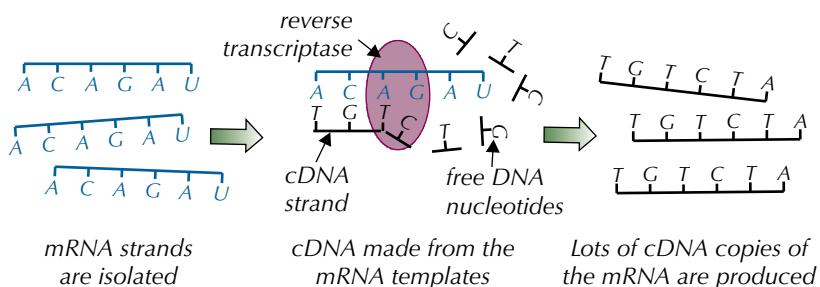


Figure 1: cDNA synthesis using reverse transcriptase.

Method 2 — using restriction endonuclease enzymes

Some sections of DNA have **palindromic sequences** of nucleotides. These sequences consist of antiparallel base pairs (base pairs that read the same in opposite directions) — see Figure 2.

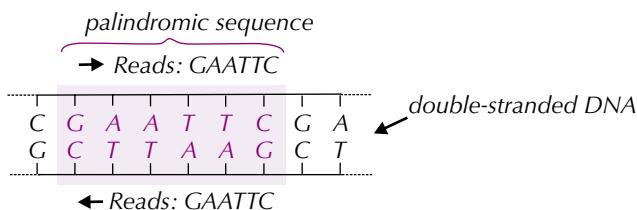


Figure 2: A palindromic DNA sequence.

Restriction endonucleases are enzymes that recognise specific palindromic sequences (known as recognition sequences) and cut (digest) the DNA at these places. Different restriction endonucleases cut at different specific recognition sequences, because the shape of the recognition sequence is complementary to the enzyme's active site.

• Examples

- The restriction endonuclease *Eco*RI cuts at GAATTC.
 - The restriction endonuclease *Hind*III cuts at AAGCTT.

If recognition sequences are present at either side of the DNA fragment you want, you can use restriction endonucleases to separate it from the rest of the DNA — see Figure 3. The DNA sample is incubated with the specific restriction endonuclease, which cuts the DNA fragment out via a hydrolysis reaction. Sometimes the cut leaves **sticky ends** — small tails of unpaired bases at each end of the fragment. Sticky ends can be used to bind (anneal) the DNA fragment to another piece of DNA that has sticky ends with complementary sequences (there's more about this on p. 479).

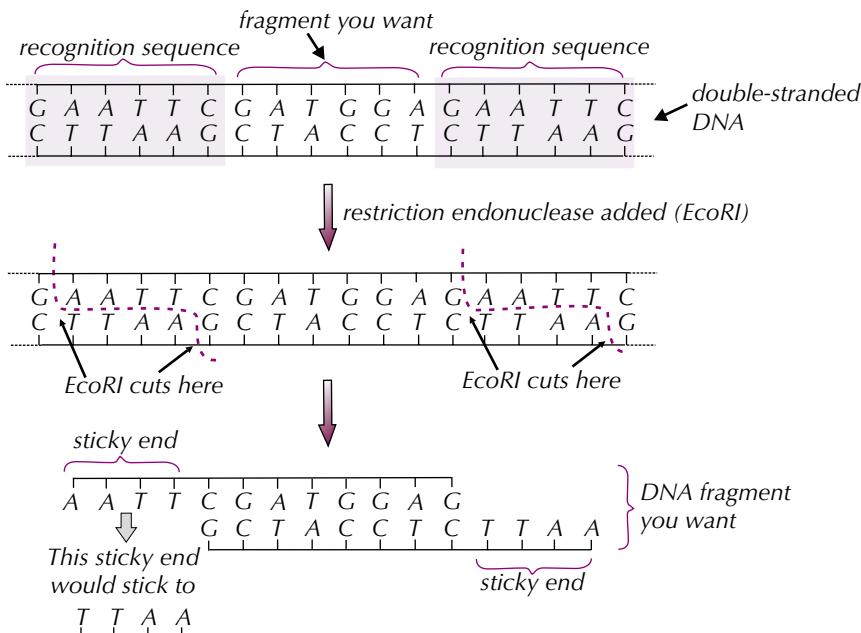
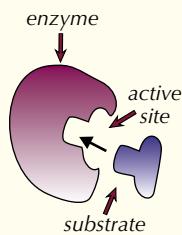


Figure 3: Using a restriction endonuclease enzyme to cut DNA.

Tip: You should remember from Topic 4 that genes contain both coding regions (exons) and non-coding regions (introns). Introns are removed from pre-mRNA to produce mRNA. Method 2 produces fragments of the whole bit of DNA you want, including the introns. But method 1 uses mRNA to make DNA, so you only get the exons.

Tip: Remember, the active site is where an enzyme's substrate binds. In this case, the recognition sequence is the substrate molecule.



Exam Tip

Make sure you use the right words to describe these processes in the exam, e.g. complementary shape not 'the same shape'.

Tip: You won't always find the same restriction enzyme site either side of the fragment you want. E.g. you might get an *Eco*RI site on one side and a *Hind*III on the other, so you'd have to incubate the DNA sample with both enzymes to cut the piece you're after.

Method 3 — using a gene machine

More recently, technology has been developed so that fragments of DNA can be synthesised from scratch, without the need for a pre-existing DNA template. Instead, a database contains the necessary information to produce the DNA fragment. This means that the DNA sequence does not have to exist naturally — any sequence can be made.

Here's how it's done:

1. The sequence that is required is designed (if one doesn't already exist).
2. The first nucleotide in the sequence is fixed to some sort of support, e.g. a bead.
3. Nucleotides are added step by step in the correct order, in a cycle of processes that includes adding protecting groups. Protecting groups make sure that the nucleotides are joined at the right points, to prevent unwanted branching.
4. Short sections of DNA called oligonucleotides, roughly 20 nucleotides long, are produced. Once these are complete, they are broken off from the support and all the protecting groups are removed. The oligonucleotides can then be joined together to make longer DNA fragments.

Practice Questions — Application

Q1 A scientist wants to produce DNA copies of a gene using some mRNA as a starting template. What enzyme will she need to do this?

Q2 Using information from the table below, describe and explain how restriction endonucleases could be used to cut this DNA sequence:

CAGGATCCTCCTTACATAGTGAATTGATGC

| Restriction endonuclease | Recognition sequence |
|--------------------------|----------------------|
| BamHI | GGATCC |
| HindIII | AAGCTT |
| EcoRI | GAATTC |

Tip: Restriction endonuclease enzymes are used a lot in gene technology to cut DNA fragments, so make sure you can answer Q2 — they'll pop up again, I promise.

Exam Tip

You could easily get asked about a restriction endonuclease you haven't heard of before — but don't panic. They all work in the same basic way, so just apply what you know to the question.

Practice Questions — Fact Recall

Q1 What is recombinant DNA technology?

Q2 Why is it possible to transfer DNA to a recipient organism of a different species?

Q3 a) What is cDNA?

- b) Describe how cDNA can be made from mRNA.
- c) Give one reason why cDNA is made in this way.

Q4 Explain what is meant by the term 'palindromic sequence'.

Q5 What are sticky ends? Why are they useful?

Q6 Describe how a gene machine can be used to synthesise a DNA fragment from scratch.

3. Amplifying DNA Fragments

Once you've got a fragment of DNA (using one of the methods on p. 476-478), you'll probably want to make more copies of it. This is done using gene cloning.

In vivo and in vitro gene cloning

Gene cloning is all about making loads of identical copies of a gene. This can be done using two different techniques:

- *In vivo* cloning — where the gene copies are made within a living organism. As the organism grows and divides, it replicates the DNA, creating multiple copies of the gene (see below).
- *In vitro* cloning — where the gene copies are made outside of a living organism using the polymerase chain reaction (PCR) (see pages 481-482).

In vivo cloning

Once you've got the DNA fragment containing the target gene you can use it for *in vivo* cloning:

Part 1 — Making recombinant DNA

The first step in *in vivo* cloning is to insert the DNA fragment into a **vector's DNA** — a vector is something that's used to transfer DNA into a cell. Vectors can be plasmids (small, circular molecules of DNA in bacteria) or bacteriophages (viruses that infect bacteria). The vector DNA is isolated and then restriction endonucleases and DNA **ligase** (an enzyme) are used to stick the DNA fragment and vector DNA together — see Figure 1.

Here's how it works:

Step 1

The vector DNA is isolated.

Step 2

The vector DNA is cut open using the same restriction endonuclease that was used to isolate the DNA fragment containing the target gene (see p. 477). This means that the sticky ends of the vector DNA are complementary to the sticky ends of the DNA fragment containing the gene.

Step 3

The vector DNA and DNA fragment are mixed together with DNA ligase. DNA ligase joins the sticky ends of the DNA fragment to the sticky ends of the vector DNA. This process is called **ligation**.

Step 4

The new combination of bases in the DNA (vector DNA + DNA fragment) is called **recombinant DNA**.

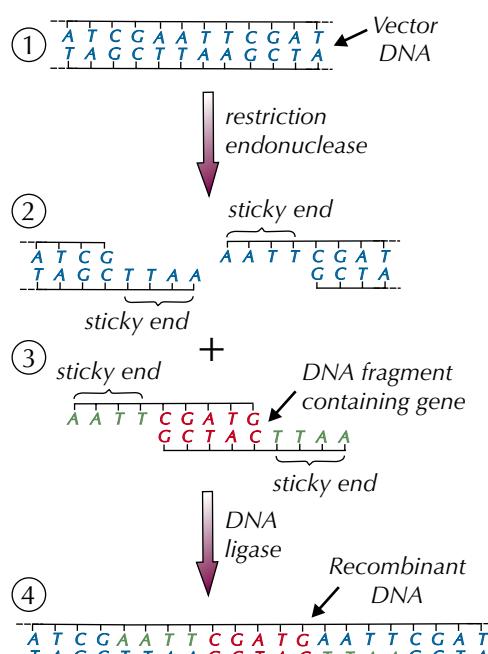


Figure 1: In vivo cloning part 1 — making recombinant DNA.

Learning Objectives:

- Know that fragments of DNA can be amplified by *in vitro* and *in vivo* techniques.
- Know that *in vivo* cloning amplifies DNA fragments using a culture of transformed host cells.
- Understand that *in vivo* cloning involves the use of restriction endonucleases and ligases to insert fragments of DNA into vectors, which are then used to transform host cells.
- Understand that in *in vivo* cloning, marker genes are used to detect genetically modified (GM) cells or organisms.
- Understand that *in vivo* cloning involves the addition of promoter and terminator regions to the fragments of DNA.
- Understand the principles of the polymerase chain reaction (PCR) as an *in vitro* method to amplify DNA fragments.

Specification Reference 3.8.4.1

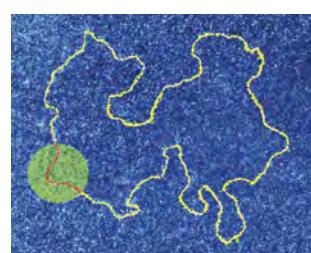


Figure 2: Recombinant plasmid DNA. The DNA fragment containing the target gene is highlighted red.

Tip: Bacteria can also be encouraged to take up DNA by electroporation — the bacteria are given a very short electric shock that's thought to create holes in the cell through which DNA can pass.

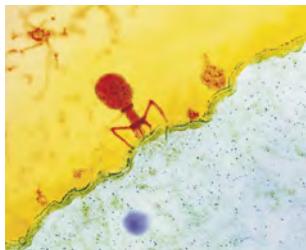


Figure 3: This isn't an alien spaceship — it's actually a bacteriophage (orange) injecting its viral DNA into an *E. coli* bacterium (blue).

Tip: Scientists will often use vector DNA that already contains a marker gene in it, then they don't have to add it in.



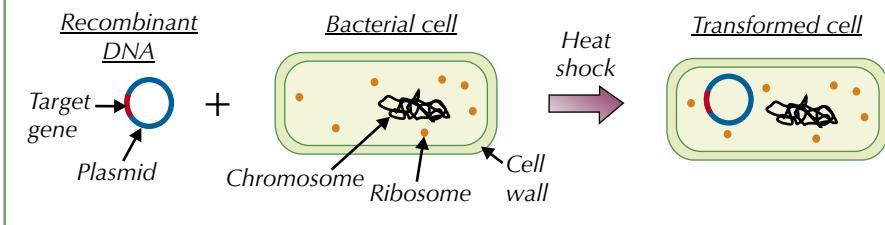
Figure 4: Fluorescing transformed bacteria colonies containing the commonly used marker gene for GFP (green fluorescent protein).

Part 2 — Transforming cells

The vector with the recombinant DNA is used to transfer the gene into cells (called **host cells**). Host cells that take up the vectors containing the gene of interest are said to be **transformed**. If a plasmid vector is used, host cells have to be persuaded to take in the plasmid vector and its DNA.

Example

Host bacterial cells are placed into ice-cold calcium chloride solution to make their cell walls more permeable. The plasmids are added and the mixture is heat-shocked (heated to around 42 °C for 1-2 minutes), which encourages the cells to take in the plasmids.



With a bacteriophage vector, the bacteriophage will infect the host bacterium by injecting its DNA into it — see Figure 3. The phage DNA (with the target gene in it) then integrates into the bacterial DNA.

Part 3 — Identifying transformed cells

Only around 5% of host cells will take up the vector and its DNA, so it's important to be able to identify which cells have been transformed.

Marker genes can be used to identify the transformed cells (see Figure 5):

Step 1

Marker genes can be inserted into vectors at the same time as the gene to be cloned. This means any transformed host cells will contain the gene to be cloned and the marker gene.

Step 2

Host cells are grown on agar plates and each cell divides and replicates its DNA, creating a colony of cloned cells. Transformed cells will produce colonies where all the cells contain the cloned gene and the marker gene.

The marker gene can code for antibiotic resistance — host cells are grown on agar plates containing the specific antibiotic, so only transformed cells that have the marker gene will survive and grow. Or the marker gene can code for fluorescence — when the agar plate is placed under a UV light only transformed cells will fluoresce.

Step 3

Identified transformed cells are allowed to grow more, producing lots and lots of copies of the cloned gene.

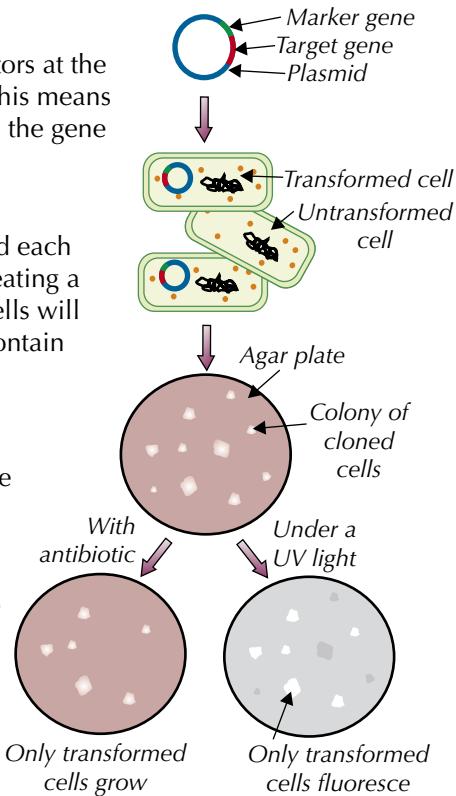


Figure 5: In vivo cloning part 3 — identifying transformed cells.

Producing proteins

If you want the transformed host cells to produce the protein coded for by the DNA fragment, you need to make sure that the vector contains specific promoter and terminator regions. Promoter regions are DNA sequences that tell the enzyme RNA polymerase where to start producing mRNA.

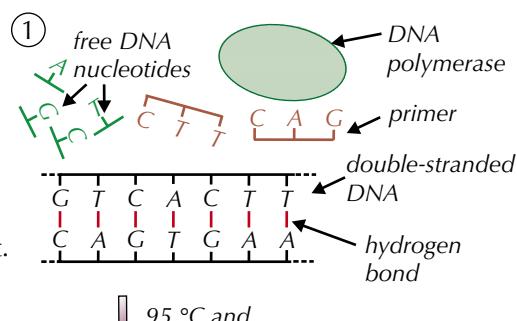
Terminator regions tell it where to stop. Without the right promoter region, the DNA fragment won't be transcribed by the host cell and a protein won't be made. Promoter and terminator regions may be present in the vector DNA or they may have to be added in along with the fragment.

In vitro cloning

DNA fragments can also be amplified using *in vitro* cloning — this is where copies of the DNA fragments are made outside of a living organism using the **polymerase chain reaction (PCR)**. PCR can be used to make millions of copies of a fragment of DNA in just a few hours. PCR has several stages and is repeated over and over to make lots of copies — see Figure 6.

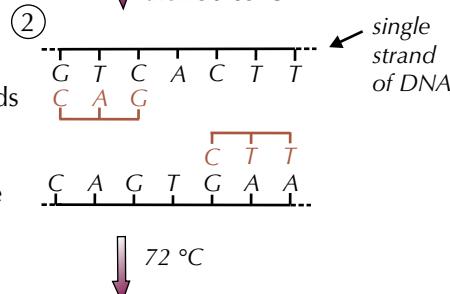
Step 1

A reaction mixture is set up that contains the DNA sample, free nucleotides, **primers** and **DNA polymerase**. Primers are short pieces of DNA that are complementary to the bases at the start of the fragment you want. DNA polymerase is an enzyme that creates new DNA strands.



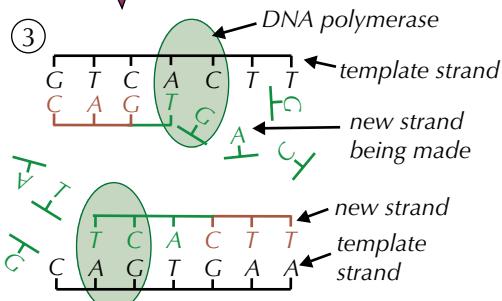
Step 2

The DNA mixture is heated to 95 °C to break the hydrogen bonds between the two strands of DNA. The mixture is then cooled to between 50 and 65 °C so that the primers can bind (anneal) to the strands.



Step 3

The reaction mixture is heated to 72 °C, so DNA polymerase can work. The DNA polymerase lines up free DNA nucleotides alongside each template strand and joins the nucleotides together. Specific base pairing means new complementary strands are formed.



Tip: We've only shown very small pieces of DNA to make the diagrams easier to follow, but real genes are much longer. (Real primers are longer too but not as big as genes.)

Tip: The DNA polymerase used in PCR is usually *Taq* polymerase. It comes from bacteria that live in hot springs, so it is able to withstand high temperatures without denaturing. Most enzymes would denature well below 95 °C.

Tip: When PCR was first developed it took a lot of time and patience as the scientist had to use multiple water baths and manually time and move the tubes from bath to bath. Nowadays the process is automated — you can buy programmable PCR machines that do almost all of the work for you.

Figure 6: First three steps of the polymerase chain reaction (PCR).

Step 4

Two new copies of the fragment of DNA are formed and one cycle of PCR is complete. The cycle starts again — the mixture is heated to 95 °C and this time all four strands (two original and two new) are used as templates.

(4)

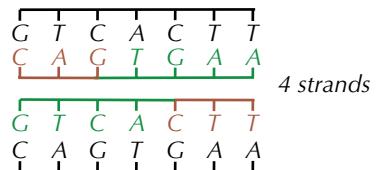


Figure 7: The final step of one cycle of the polymerase chain reaction (PCR).



Figure 8: Scientist using a programmable PCR machine.

As shown below, each PCR cycle doubles the amount of DNA, e.g. 1st cycle = $2 \times 2 = 4$ DNA fragments, 2nd cycle = $4 \times 2 = 8$ DNA fragments, 3rd cycle = $8 \times 2 = 16$ DNA fragments, and so on.

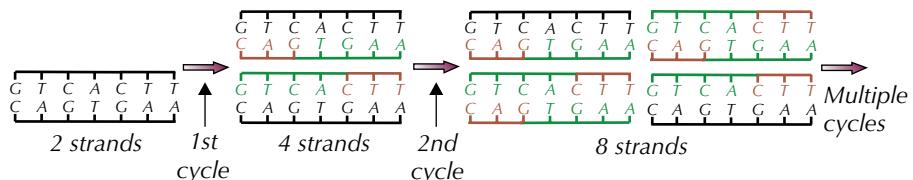
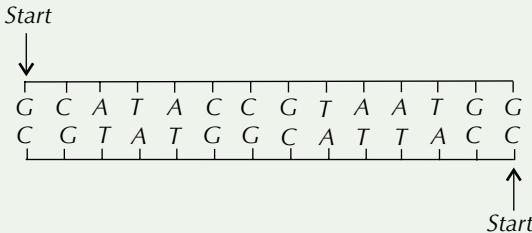


Figure 9: DNA doubling during each cycle of PCR.

Practice Questions — Application

- Q1 A scientist is studying the role of a protein in cancer progression. He used *in vivo* cloning to transform some *E. coli* cells with recombinant DNA containing the gene that codes for the protein. He then grew the cells on an agar plate containing penicillin.
- A DNA fragment containing the target gene is made using restriction endonucleases. Describe and explain how the recombinant DNA is produced using this fragment.
 - Explain why you think the cells have been grown on an agar plate containing penicillin.
- Q2 The following DNA fragment is being copied using PCR. The arrows mark the start of each DNA strand.



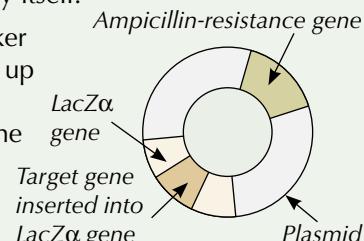
- The scientist carrying out the PCR uses primers that are four bases long. Give the sequences of the primers he will need to use to copy the DNA fragment.
- The scientist carries out six cycles of PCR. How many single strands of DNA will he have once the six cycles are complete?

Q3 Read the passage below and answer the questions that follow.

The LacZ gene is found in *E.coli*. It codes for an enzyme called β -galactosidase. β -galactosidase breaks down the colourless substance X-gal into a blue pigment.

LacZ α and LacZ Ω are mutated versions of the LacZ gene. Each one codes for a protein that forms part of the β -galactosidase enzyme. When the two proteins are produced in the same cell, they assemble to form a fully-functional β -galactosidase enzyme. Neither protein works as the enzyme by itself.

LacZ α and LacZ Ω can be used as marker genes to test whether *E.coli* have taken up recombinant DNA. The target gene is inserted into the middle of a LacZ α gene on bacterial plasmids (see diagram on the right). The plasmids also contain a gene for ampicillin-resistance.



The plasmids are taken up by *E.coli* containing a copy of the LacZ Ω gene. The *E.coli* are then cultured on agar plates containing X-gal and ampicillin.

- What is the role of the bacterial plasmids?
- Explain why the plasmids contain an ampicillin-resistance gene.
- E.coli* that have taken up plasmids containing the target gene will be white. *E.coli* containing plasmids without the target gene will be blue. Explain why this is the case.

Exam Tip

Make sure you read any information you get given in the exam very carefully. It's there for a reason — to help you answer the question.

Tip: Inserting a target gene into another gene disrupts the transcription and translation of that gene.

Practice Questions — Fact Recall

- How is *in vivo* cloning different to *in vitro* cloning?
- Explain what is meant by the term 'vector'.
- Give an example of a vector used in *in vivo* cloning.
- Describe the role of DNA ligase in *in vivo* cloning.
- In *in vivo* cloning, what is a host cell?
- What does it mean when a cell is described as being 'transformed'?
- Explain the importance of identifying transformed cells in *in vivo* cloning.
- What are marker genes used for?
- What specific sequences might you need to add to vector DNA to ensure the transformed host cell will produce the protein that is coded for by the DNA fragment?
- Name the process that *in vitro* cloning uses.
- a) In *in vitro* cloning, what should the reaction mixture contain?
b) Describe the process that happens once the mixture is set up.

Exam Tip

Make sure you know the differences between *in vivo* and *in vitro* cloning so you don't get confused between the two processes. They may have similar names but they're actually very different.

Learning Objectives:

- Be able to interpret information relating to the use of recombinant DNA technology.
- Be able to evaluate the ethical, financial and social issues associated with the use and ownership of recombinant DNA technology in agriculture, in industry and in medicine.
- Be able to balance the humanitarian aspects of recombinant DNA technology with the opposition from environmentalists and anti-globalisation activists.

Specification Reference 3.8.4.1

Tip: Transformed organisms are also known as genetically engineered or genetically modified (GM) organisms.



Figure 1: Genetically engineered mice. The jellyfish gene that codes for green fluorescent protein has been inserted into the mice so they fluoresce.

4. Recombinant DNA Technology

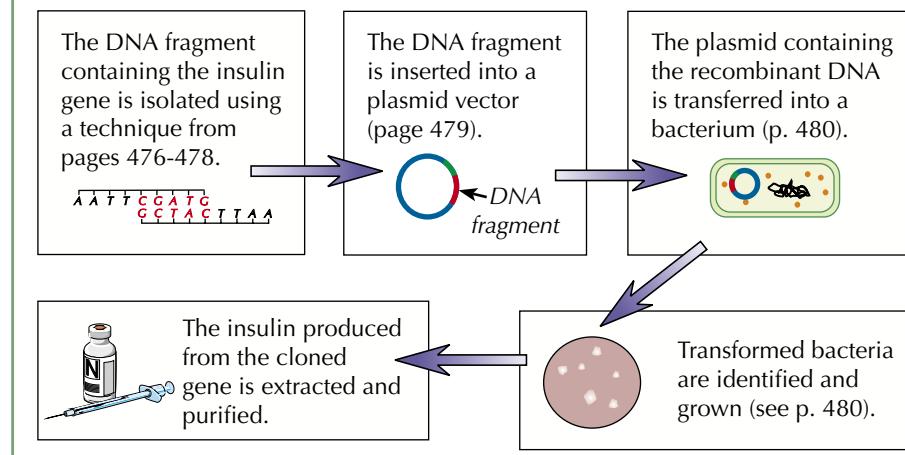
You get stories on genetically engineered crops and food popping up a lot in newspapers. Let's start with what genetic engineering really is.

Transformed organisms

Microorganisms, plants and animals can all be transformed using recombinant DNA technology. This is called genetic engineering. Transformed microorganisms can be made using the same technology as *in vivo* cloning (see pages 479-480).

Example

Foreign DNA can be inserted into microorganisms to produce the protein insulin. Here's how:



Transformed plants can also be produced — a gene that codes for a desirable protein is inserted into a plasmid. The plasmid is added to a bacterium and the bacterium is used as a vector to get the gene into the plant cells. If the right promoter region has been added along with the gene (see p. 481), the transformed cells will be able to produce the desired protein.

Transformed animals can be produced too — a gene that codes for a desirable protein can be inserted into an early animal embryo or into the egg cells of a female. If the gene is inserted into a very early embryo, all the body cells of the resulting transformed animal will end up containing the gene. Inserting it into the egg cells means that when the female reproduces, all the cells of her offspring will contain the gene.

Promoter regions that are only activated in specific cell types can be used to control exactly which of an animal's body cells the protein is produced in. If the protein is only produced in certain cells, it can be harvested more easily. Producing the protein in the wrong cells could also damage the organism.

The recombinant DNA technology debate

Some people have concerns about transformed organisms (see pages 485-486). But producing transformed organisms can benefit humans in lots of ways. You need to understand both sides of the recombinant DNA technology debate — then you can balance the humanitarian benefits with opposing views from environmentalists and anti-globalisation activists.

The benefits of transformed organisms

Humanitarians believe that using recombinant technology will benefit people in lots of different ways:

In agriculture

Agricultural crops can be transformed so that they give higher yields or are more nutritious. This means these plants can be used to reduce the risk of famine and malnutrition.

Example

Golden Rice is a variety of transformed rice. It contains one gene from maize and one gene from a soil bacterium, which together enable the rice to produce beta-carotene. The beta-carotene is used by our bodies to produce vitamin A. Golden Rice is being developed to reduce vitamin A deficiency in areas where there's a shortage of dietary vitamin A, e.g. south Asia, Africa. Vitamin A deficiency is a big problem in these areas (up to 500 000 children per year worldwide go blind due to vitamin A deficiency).

Crops can also be transformed to have resistance to pests or droughts. Pest-resistant crops need fewer pesticides, which reduces costs and any environmental problems associated with using the chemicals.

Drought-resistant crops can survive in drought-prone areas with little water.

In industry

Industrial processes often use enzymes (biological catalysts). These enzymes can be produced from transformed organisms, so they can be produced in large quantities for less money, reducing costs.

Example

Chymosin (or rennin) is an enzyme used in cheese-making. It used to be made from rennet (a substance produced in the stomach of cows), but it can now be produced by transformed organisms. This means it can be made in large quantities, relatively cheaply and without killing any cows, making some cheese suitable for vegetarians.

In medicine

Many drugs and vaccines are produced by transformed organisms using recombinant DNA technology.

Example

Insulin is used to treat Type 1 diabetes and used to come from animal (cow, horse or pig) pancreases. This insulin wasn't human insulin though, so it didn't work quite as well. Human insulin is now made from transformed microorganisms, using a cloned human insulin gene (see previous page).

Drugs made using recombinant DNA technology can be produced quickly, cheaply and in large quantities. This could make them more affordable and so available to more people.

Exam Tip

Transformed organisms (microorganisms, plants and animals) can be used in a variety of ways. You need to be able to interpret information about how they are used.



Figure 2: Genetically engineered (transformed) Golden Rice (right) compared to normal white rice (left).

Tip: Recombinant DNA technology has the potential to be used in gene therapy to treat human diseases (see page 487).

Tip: Transformed crops could be used to make vaccines in areas where refrigeration isn't available (vaccines usually need to be stored in fridges). This would make the vaccines available to more people.

The concerns about transformed organisms

Some people have ethical, financial and social concerns about the use of recombinant DNA technology. These people include anti-globalisation activists (who oppose globalisation, e.g. the growth of large multinational companies at the expense of smaller ones). Some environmentalists also have concerns about the possible environmental effects of the technology.

Tip: Biodiversity describes the variety of living organisms in an area. Monoculture reduces biodiversity by reducing the number of plant species in an area. This in turn reduces the number of other species, e.g. insects, that the area can support.

In agriculture

Farmers might plant only one type of transformed crop (this is called monoculture). This could make the whole crop vulnerable to the same disease because the plants are genetically identical. Environmentalists are also concerned about monocultures reducing biodiversity, as this could damage the environment.

Some people are concerned about the possibility of 'superweeds' — weeds that are resistant to herbicides. These could occur if transformed crops interbreed with wild plants. There could then be an uncontrolled spread of recombinant DNA, with unknown consequences.

Organic farmers can have their crops contaminated by wind-blown seeds from nearby genetically modified crops. This means they can't sell their crop as organic and may lose their income.

In industry

Without proper labelling, some people think they won't have a choice about whether to consume food made using genetically engineered organisms. Some people are worried that the process used to purify proteins (from genetically engineered organisms) could lead to the introduction of toxins into the food industry.

A few, large biotechnology companies control some forms of genetic engineering. As the use of this technology increases, these companies get bigger and more powerful. This may force smaller companies out of business, e.g. by making it harder for them to compete. Anti-globalisation activists are against this.

In medicine

Companies who own genetic engineering technologies may limit the use of technologies that could be saving lives. Also, some people worry that this technology could be used unethically, e.g. to make designer babies (babies that have characteristics chosen by their parents). This is currently illegal though.

Recombinant DNA technology also creates ownership issues.

Examples

- There is some debate about who owns genetic material from humans once it has been removed from the body — the donor or the researcher. Some people argue that the individual holds the right to their own genetic information. However, others argue that value is created by the researcher who uses it to develop a medicine or in diagnosis.
- A small number of large corporations own patents to particular seeds. They can charge high prices, sometimes including a 'technology fee', and can require farmers to repurchase seeds each year. If non-GM crops are contaminated by GM crops, farmers can be sued for breaching the patent law.

Practice Questions — Application

A large agricultural company's research and development department have created transformed soybean plants that are resistant to a certain herbicide. The resistance gene was isolated from bacteria.

- Q1 Explain how the transformed soybean plant could have been created.
- Q2 Suggest how the transformed soybean plant may benefit humans.
- Q3 Why might some people oppose the use of this transformed plant?

5. Gene Therapy

Recombinant DNA technology could also be used to treat human diseases. This is known as gene therapy.

How does gene therapy work?

Gene therapy involves altering the defective genes (mutated alleles) inside cells to treat genetic disorders and cancer. How you do this depends on whether the disorder is caused by a mutated dominant allele or two mutated recessive alleles.

- If it's caused by two mutated recessive alleles you can add a working dominant allele to make up for them — you 'supplement' the faulty ones.
- If it's caused by a mutated dominant allele you can 'silence' the dominant allele (e.g. by sticking a bit of DNA in the middle of the allele so it doesn't work any more).

Both of these processes involve inserting a DNA fragment into the person's original DNA. Just like in recombinant DNA technology, you need a vector to get the DNA into the cell (see page 479). A range of different vectors can be used, e.g. altered viruses, plasmids or liposomes (spheres made of lipid).

The two types of gene therapy

There are two types of gene therapy:

Somatic therapy

This involves altering the alleles in body cells, particularly the cells that are most affected by the disorder.

Example

Cystic fibrosis (CF) is a genetic disorder that's very damaging to the respiratory system, so somatic therapy for CF targets the epithelial cells lining the lungs.

Somatic therapy doesn't affect the individual's sex cells (sperm or eggs) though, so any offspring could still inherit the disease.

Germ line therapy

This involves altering the alleles in the sex cells. This means that every cell of any offspring produced from these cells will be affected by the gene therapy and they won't suffer from the disease. Germ line therapy in humans is currently illegal though.

Learning Objective:

- Be able to relate recombinant DNA technology to gene therapy.

Specification Reference 3.8.4.1

Tip: If you can't remember the difference between dominant and recessive alleles, check out page 379.

Tip: Gene therapy isn't being used widely yet, but there is a form of somatic gene therapy available to treat some people with a genetic disease called LPLD. Treatments for other diseases, such as cystic fibrosis, are undergoing clinical trials.

Tip: Sex cells are the gametes — eggs and sperm. Body cells are all the rest, e.g. skin cells, liver cells, heart cells, etc.

Ethical issues surrounding gene therapy

There are also many ethical issues associated with gene therapy. For example, some people are worried that the technology could be used in ways other than for medical treatment, such as for treating the cosmetic effects of aging. Other people worry that there's the potential to do more harm than good by using the technology (e.g. risk of overexpression of genes — gene produces too much of the missing protein).

Practice Questions — Fact Recall

Q1 What does gene therapy involve?

Q2 What is the difference between somatic and germ line therapy?

Learning Objectives:

- Understand the use of labelled DNA probes and DNA hybridisation to locate specific alleles of genes.
- Understand the use of labelled DNA probes that can be used to screen patients for heritable conditions, drug responses or health risks.
- Understand the use of screening information in genetic counselling and personalised medicine.
- Be able to evaluate information relating to screening individuals for genetically determined conditions and drug responses.

Specification Reference 3.8.4.2

Tip: If a radioactively labelled probe is used, the fragments are transferred to X-ray film. If the gene and probe are present, a shadow will form on the film.

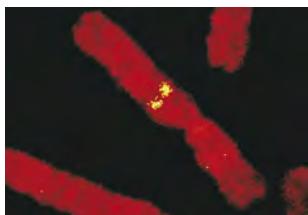


Figure 3: Human chromosomes (red) with DNA probes (yellow) hybridised to complementary base sequences.

6. Gene Probes and Medical Diagnosis

To produce a DNA probe, you first need to sequence the allele that you want to screen for (see page 474). You then use PCR (see p. 481) to produce multiple complementary copies of part of the allele — these are the probes.

Locating alleles using DNA probes

DNA probes can be used to locate specific alleles of genes (e.g. on chromosomes) or to see if a person's DNA contains a mutated allele that causes a genetic disorder. DNA probes are short strands of DNA — see Figure 1. They have a specific base sequence that's complementary to the base sequence of part of a target allele (the allele you're looking for, e.g. an allele that causes a genetic disorder). This means a DNA probe will bind (hybridise) to the target allele if it's present in a sample of DNA.

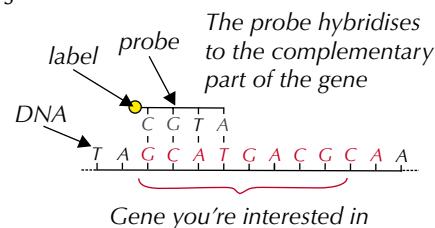


Figure 1: A DNA probe.

A DNA probe also has a label attached, so that it can be detected. The two most common labels are a radioactive label (detected using X-ray film) or a fluorescent label (detected using UV light). Figure 2 and the text below explain how fluorescently labelled probes are used:

Step 1

A sample of DNA is digested into fragments using restriction enzymes (see page 477) and separated using electrophoresis (see page 492).

Step 2

The separated DNA fragments are then transferred to a nylon membrane and incubated with a fluorescently labelled DNA probe. If the allele is present, the DNA probe will bind (hybridise) to it.

Step 3

The membrane is then exposed to UV light and if the gene is present there will be a fluorescent band. E.g. in this case, the DNA in fragment X contains the target allele.

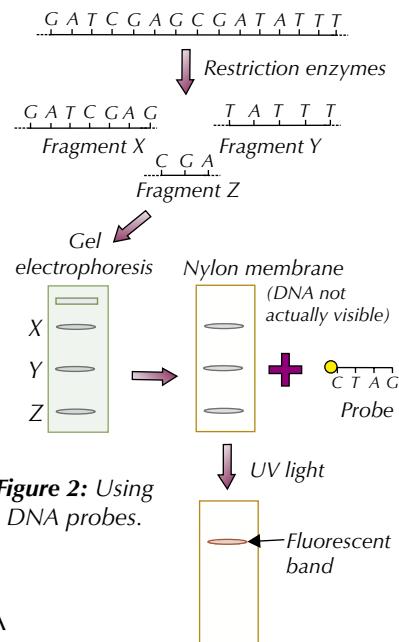


Figure 2: Using DNA probes.

Screening for multiple genes

The probe can be used as part of a DNA **microarray**, which can screen for lots of different genes at the same time. A DNA microarray is a glass slide (see Figures 4 and 5 on the next page) with microscopic spots of different DNA probes attached to it in rows.

A sample of fluorescently labelled human DNA is washed over the array. If the labelled human DNA contains any DNA sequences that match any of the probes, it will stick to the array. So this means you can screen the DNA for lots of different mutated genes at the same time. The array is washed, to remove any fluorescently labelled DNA that hasn't stuck to it, and then visualised under UV light. Any labelled DNA attached to a probe will show up (fluoresce) — see Figure 4. Any spot that fluoresces means that the person's DNA contains that specific allele. E.g. if the probe is for a mutated allele that causes a genetic disorder, this person has the allele.

Tip: Microarrays aren't just used to diagnose genetic diseases. Researchers can use them to analyse other DNA samples, or even to analyse mRNA samples.

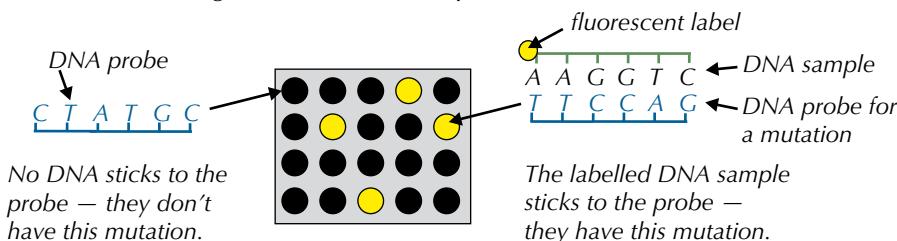


Figure 4: Diagram of a DNA microarray.

Uses of screening with DNA probes

Screening using DNA probes has lots of uses. For example:

- It can be used to help identify inherited conditions.

Examples

- Huntington's disease is an inherited condition that affects the nervous system and does not usually start to display symptoms until a person is aged between 30 and 50. People with a family history of the disease may choose to be screened for the mutated allele to find out if they have inherited it.
- The NHS offers to screen all newborn babies for the inherited disorder cystic fibrosis (which can cause breathing and digestive difficulties) so that treatment for the condition can begin as soon as possible.
- It can be used to help determine how a patient will respond to specific drugs.

Example

Breast cancer can be caused by a mutation in the HER2 proto-oncogene and treated with the drug Herceptin® (see page 453). Herceptin® is only effective against this type of breast cancer because it targets a specific receptor. Screening for this particular mutation helps determine whether Herceptin® will be a useful treatment or not.

- It can also be used to help identify health risks.

Example

Inheriting particular mutated alleles increases your risk of developing certain types of cancer (although it doesn't make it certain that you'll develop cancer). If a person knows they have these alleles, it might help them make choices that could reduce the risk of the disease developing (see next page).

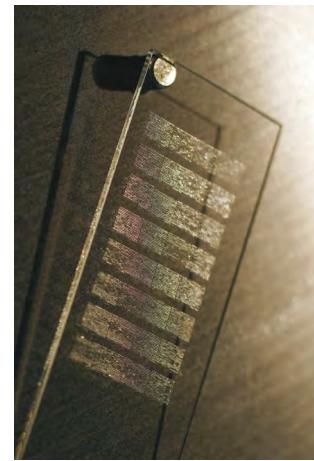


Figure 5: A DNA microarray.

Tip: You need to be able to evaluate information about screening for inherited conditions and people's responses to drugs.

However, some people are concerned that genetic screening may lead to discrimination by insurance companies and employers if people are known to have a high risk of developing a condition.

Genetic counselling

The results of screening can be used for genetic counselling. Genetic counselling is advising patients and their relatives about the risks of genetic disorders. It involves advising people about screening (e.g. looking for mutated alleles if there's a family history of cancer) and explaining the results of a screening. Screening can help to identify if someone is the carrier of a mutated allele, the type of mutated allele they're carrying (indicating the type of genetic disorder or cancer) and the most effective treatment. If the results of a screening are positive (an individual has the mutation) then genetic counselling is used to advise the patient on the options of prevention or treatment available.

Example 1

A woman with a family history of breast cancer may have genetic counselling to help her decide whether or not to be screened for known mutations that can lead to breast cancer, e.g. a mutation in the BRCA1 tumour suppressor gene (see page 453). If she is screened and the result is positive, genetic counsellors might explain to the woman what her lifetime chance of developing breast cancer is (a woman with the mutated BRCA1 gene has around a 50 to 85% chance of developing breast cancer in her lifetime). Counselling could also help the woman to decide if, for example, she wants to take surgical steps to reduce the risk of breast cancer developing (by having a mastectomy).

Tip: A carrier is a person with an allele that is not expressed in their phenotype but that can be passed on to offspring — see p. 386.

Example 2

Sickle-cell anaemia is a recessive genetic disorder caused by a mutation in the haemoglobin gene. A couple who are both carriers of the sickle-cell allele may like to have kids. They may undergo genetic counselling to help them understand their chances of having a child with sickle-cell anaemia (one in four). Genetic counselling also provides unbiased advice on the possibility of having IVF and screening their embryos for the allele, so embryos without the mutation are implanted in the womb. It could also provide information on the help and drugs available if they have a child with sickle-cell anaemia.

Personalised medicine

The results of screening can also be used in personalised medicine. Your genes determine how your body responds to certain drugs. Different people respond to the same drug in different ways — which makes certain drugs more effective for some people than others. This is where personalised medicines come in. Personalised medicines are medicines that are tailored to an individual's DNA. The theory is that if doctors have your genetic information, they can use it to predict how you will respond to different drugs and only prescribe the ones that will be most effective for you.

Practice Questions — Application

A couple's first child has been born showing symptoms associated with a variety of genetic disorders. Scientists decide to screen the child to determine which specific genetic mutation his DNA contains.

- Q1 Describe the process that should be used to screen the child.
- Q2 The child is eventually diagnosed with a rare recessive genetic disorder. Explain why the couple might have genetic counselling.

7. Genetic Fingerprinting

As well as actual fingerprints, forensic scientists can now use genetic fingerprinting to identify people by their DNA.

The principles of genetic fingerprinting

Not all of an organism's genome (all the genetic material in an organism) codes for proteins. Some of the genome consists of variable number tandem repeats (VNTRs) — base sequences that don't code for proteins and repeat next to each other over and over (sometimes thousands of times), e.g. CATGCATGCATG is a repeat of the non-coding base sequence CATG.

The number of times these sequences are repeated differs from person to person, so the length of these sequences in nucleotides differs too. E.g. a four nucleotide sequence might be repeated 12 times in one person giving 48 nucleotides (12×4), but repeated 16 times in another person giving 64 nucleotides (16×4).

The repeated sequences occur in lots of places in the genome. The number of times a sequence is repeated (and so the number of nucleotides) at different places in the genome can be compared between individuals — this is called **genetic fingerprinting**. The probability of two individuals having the same genetic fingerprint is very low because the chance of two individuals having the same number of VNTRs at each place they're found in DNA is very low.

Producing genetic fingerprints

So genetic fingerprints can be compared between different individuals. Now you need to know how one is made.

Step 1 — PCR is used to make DNA fragments

A sample of DNA is obtained, e.g. from a person's blood, saliva, etc. PCR (see page 481) is used to make many copies of the areas of DNA that contain the VNTRs — see Figure 1. Primers are used that bind to either side of these repeats and so the whole repeat is amplified (copied many times). Different primers are used for each position under investigation. You end up with DNA fragments where the length (in nucleotides) corresponds to the number of repeats the person has at each specific position, e.g. one person may have 80 nucleotides, another person 120. A fluorescent tag is added to all the DNA fragments (usually to the primers) so they can be viewed under UV light (see next page).

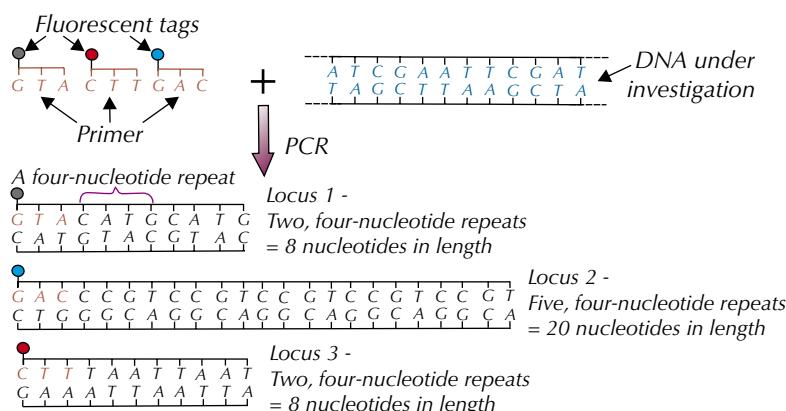


Figure 1: DNA fragments are made for fingerprint analysis by PCR.

Learning Objectives:

- Know that an organism's genome contains many variable number tandem repeats (VNTRs).
- Know that the probability of two individuals having the same VNTRs is very low.
- Understand the technique of genetic fingerprinting in analysing DNA fragments that have been cloned by PCR, and its use in determining genetic relationships and in determining the genetic variability within a population.
- Be able to explain the biological principles that underpin genetic fingerprinting techniques.
- Be able to interpret data showing the results of gel electrophoresis to separate DNA fragments.
- Be able to explain why scientists might use genetic fingerprinting in the fields of forensic science, medical diagnosis and animal and plant breeding.

Specification Reference 3.8.4.3

Tip: A locus (plural, loci) is the fixed position of a gene on a chromosome (see page 378).

Tip: Fragments move through the gel in order of length, so longer fragments stay towards the top (-ve) end and shorter fragments move further down (towards the +ve end).



Figure 2: A scientist loading a DNA sample into a gel.

Tip: The positive electrode is called the anode and the negative electrode is called the cathode.

Tip: Gels are also used to separate RNA by length or proteins according to size. (And they can be run vertically in slightly different equipment too.)

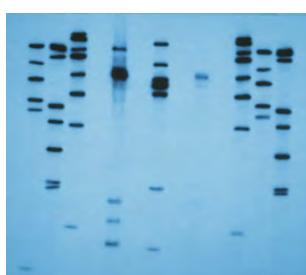


Figure 4: A genetic fingerprint.

Step 2 — Separation of the DNA fragments by gel electrophoresis

To separate out DNA fragments, the DNA mixture is placed into a well in a slab of gel and covered in a buffer solution that conducts electricity — see Figure 2 (Side view). An electrical current is passed through the gel — DNA fragments are negatively charged, so they move towards the positive electrode at the far end of the gel. Shorter DNA fragments move faster and travel further through the gel, so the DNA fragments separate according to length. This produces a pattern of bands — see Figure 3 (View of gel from above).

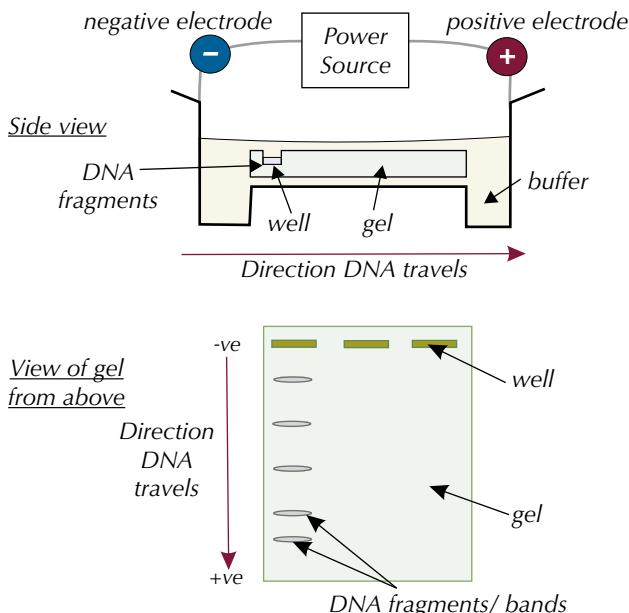


Figure 3: DNA fragments are separated by gel electrophoresis.

Step 3 — Analysis of the genetic fingerprints

After the gel has been running long enough, the equipment is turned off and the gel is placed under a UV light. Under the UV light the DNA fragments can be seen as bands. These bands make up the genetic fingerprint — see Figure 5. A DNA ladder may have been added to one well — this is a mixture of DNA fragments of known length that allows you to work out the length of the other bands on the gel. Two genetic fingerprints can be compared, e.g. if both fingerprints have a band at the same location on the gel it means they have the same number of nucleotides and so the same number of VNTRs at that place — it's a match.

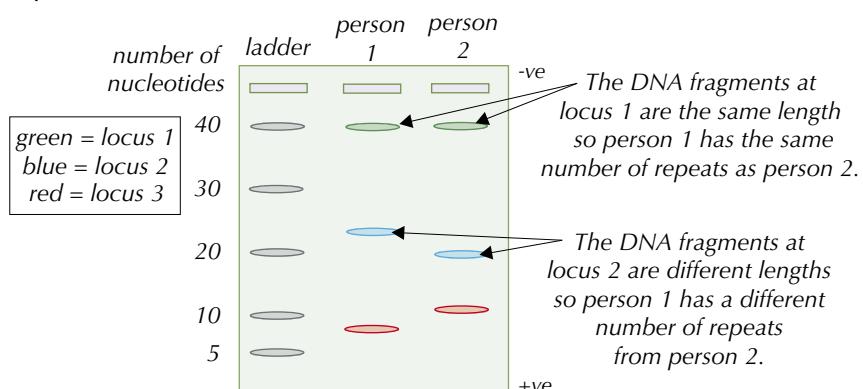


Figure 5: Diagram showing a genetic fingerprint.

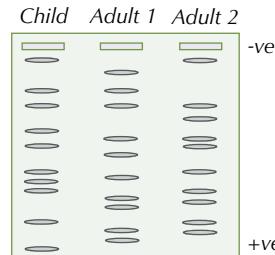
Uses of genetic fingerprinting

1. Determining genetic relationships

We inherit VNTR base sequences from our parents. Roughly half of the sequences come from each parent. This means the more bands on a genetic fingerprint that match, the more closely related (genetically similar) two people are.

Example

Paternity tests are used to determine the biological father of a child by comparing genetic fingerprints. If lots of bands match, then that person is most probably the child's father. The higher the number of places in the genome compared, the more accurate the test result. The gel on the right shows that Adult 2 is most likely the father, as six out of ten bands match.



Tip: Roughly half the bands will match in a paternity test as we inherit half our DNA from our mum and half from our dad.

Genetic fingerprinting can also be used to look at much wider ranging genetic relationships, e.g. to see if a population of black bears found in Virginia is descended from a population in Canada or Alaska. The idea is still the same — the more bands the populations have in common, the more closely related they are.

Sometimes you might be interested in tracing only the male or female line of descent. To look at the female line of descent you need to look at DNA in mitochondria. This is because in humans and most other organisms, mitochondrial DNA (mtDNA) is only inherited from your mum. If you're after the male side, you need to look at Y chromosome DNA, as only men have a Y chromosome.

Tip: Comparing mtDNA to see how closely related species are is used a lot in phylogenetics (the study of the evolution of organisms).

2. Determining genetic variability within a population

The greater the number of bands that don't match on a genetic fingerprint, the more genetically different individuals are. This means you can compare the number of repeats at several places in the genome for a population to find out how genetically varied that population is. E.g. the more the number of repeats varies at several places, the greater the genetic variability within a population.

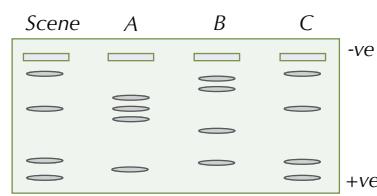
3. In forensic science

Forensic scientists use genetic fingerprinting to compare samples of DNA collected from crime scenes (e.g. DNA from blood, semen, skin cells, saliva, hair etc.) to samples of DNA from possible suspects, which could link them to crime scenes. The DNA is isolated from all the collected samples (from the crime scene and from the suspects). Each sample is replicated using PCR (see p. 481). The PCR products are run on an electrophoresis gel and the genetic fingerprints produced are compared to see if any match. If the samples match, it links a person to the crime scene.

Tip: PCR is used to amplify the areas of DNA that contain the repeated sequences, so enough is produced for them to be seen on the gel.

Example

This gel shows that the genetic fingerprint from suspect C matches that from the crime scene, linking them to the crime scene. All four bands match, so suspect C has the same number of repeats (nucleotides) at four different places.



Tip: In fingerprint analysis in the UK, the results from ten different loci (plural for locus) are analysed. The chances of two fingerprints matching by chance is at least 1 in 1000 million.

Tip: The type of genetic fingerprinting used in medical diagnosis is slightly different to the one described on pages 491-492, but don't worry — you just need to know why genetic fingerprinting is important for medical diagnosis.

Tip: Genetic disorders and cancer are both caused by mutations in DNA — see page 443 for more.

Tip: A specific mutation can be found using gene probes and sequencing (see p. 488-489).

4. For medical diagnosis

In medical diagnosis, a genetic fingerprint can refer to a unique pattern of several alleles. It can be used to diagnose genetic disorders and cancer. It's useful when the specific mutation isn't known or where several mutations could have caused the disorder, because it identifies a broader, altered genetic pattern.

Example 1

Preimplantation genetic haplotyping (PGH) screens embryos created by IVF for genetic disorders before they're implanted into the uterus. The faulty regions of the parents' DNA are used to produce genetic fingerprints, which are compared to the genetic fingerprint of the embryo. If the fingerprints match, the embryo has inherited the disorder and so it is not implanted. It can be used to screen for cystic fibrosis, Huntington's disease, etc.

Example 2

Genetic fingerprinting can be used to diagnose sarcomas (types of tumour). Conventional methods of identifying a tumour (e.g. biopsies) only show the physical differences between tumours. Now the genetic fingerprint of a known sarcoma (e.g. the different mutated alleles) can be compared to the genetic fingerprint of a patient's tumour. If there's a match (i.e. the mutated alleles are the same), the sarcoma can be specifically diagnosed and the treatment can be targeted to that specific type (see page 453).

5. In animal and plant breeding

Genetic fingerprinting can be used on animals and plants to prevent inbreeding, which decreases the gene pool (the number of different alleles in a population, see p. 400). Inbreeding can lead to an increased risk of genetic disorders, leading to health, productivity and reproductive problems. Since genetic fingerprinting can be used to identify how closely related individuals are (see previous page), it can be used to identify the least related individuals in a population so that we can breed them together.

Genetic fingerprinting can also be used by animal breeders to prove pedigree (who an animal's parents and descendants are). Animals with a good pedigree will sell for more money. E.g. the offspring of Crufts or Grand National winners can sell for a lot of money if you can prove their pedigree.

Practice Questions — Application

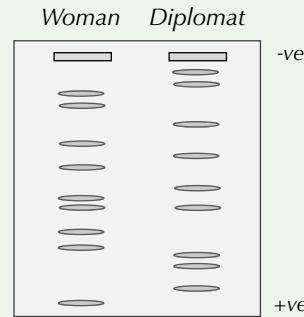
A young woman has come forward claiming to be the long lost daughter of a wealthy diplomat. Scientists have used genetic fingerprinting to produce the gel shown below.

Q1 Explain how the structure of an organism's genome allows a genetic fingerprint to be made.

Q2 For what purpose during the genetic fingerprinting procedure would the scientists have used the following:

- PCR,
- gel electrophoresis?

Q3 Do you believe the woman is the daughter of the diplomat? Explain your answer.



Exam Tip

In the exams, you could get a question about genetic fingerprinting in any of a huge range of contexts. So make sure you understand and can explain how it works, and are able to describe all its possible uses.

Section Summary

Make sure you know...

- That genome projects have sequenced the genomes of a wide range of organisms, including humans.
- That sequencing the genome of a simpler organism allows us to determine all the proteins that it can make (its proteome) and that this is useful in identifying antigens for creating vaccines.
- That more complex organisms have lots of non-coding DNA and complex regulatory genes, which make it difficult to translate the sequenced genomes into the proteomes.
- That sequencing methods are continually being improved and are now automated.
- That recombinant DNA technology involves the transfer of fragments of DNA from one organism to another and that the transferred DNA can be translated in the transformed organism due to the universal nature of the genetic code.
- That fragments of DNA can be made from mRNA using reverse transcriptase and that this DNA is called complementary DNA (cDNA).
- That fragments of DNA can also be isolated using restriction endonuclease enzymes. These enzymes recognise and cut DNA at different, specific palindromic sequences of nucleotides.
- That fragments of DNA can be made in a 'gene machine'.
- That *in vitro* and *in vivo* cloning can be used to amplify DNA fragments.
- That *in vivo* cloning is when copies of genes are made inside a living organism.
- That *in vivo* cloning involves creating recombinant DNA (using restriction endonucleases and ligases), producing transformed cells, and then identifying and growing those cells.
- That in *in vivo* cloning, marker genes are added so that genetically modified cells can be identified.
- That in *in vivo* cloning, you need to add promoter and terminator regions to the fragments of DNA.
- That *in vitro* cloning is when copies of genes are made outside of a living organism using the polymerase chain reaction (PCR).
- That recombinant DNA technology (genetic engineering) can be used to produce transformed organisms and the benefits of this for agriculture, industry and medicine.
- Some of the ethical, financial and social concerns about the use of recombinant DNA technology in agriculture, industry and medicine, and be able to evaluate these concerns.
- How to interpret information relating to the use of recombinant DNA technology and be able to balance the benefits of genetic engineering with the concerns and opposition from various groups.
- How recombinant DNA technology might be used in gene therapy to alter defective genes inside body cells (somatic gene therapy) or sex cells (germ line gene therapy).
- That DNA probes are short strands of labelled DNA that can be used to locate certain sequences of DNA (e.g. target alleles). A DNA probe is complementary to its target allele, so will hybridise (bind) to it.
- That DNA probes can be used to screen patients for heritable conditions, drug responses or health risks, and any information gained can be used in genetic counselling and to develop personalised medicine.
- That genomes contain variable number tandem repeats (VNTRs) that can be used in genetic fingerprinting, as the probability of two individuals having the same VNTRs is very low.
- That genetic fingerprinting involves using PCR to clone DNA fragments from a sample of DNA, running these fragments on an electrophoresis gel to separate them according to size, and then comparing the length of the fragments against other DNA samples.
- How to analyse genetic fingerprinting gels by looking at the pattern of bands produced.
- That genetic fingerprinting can be used to determine genetic relationships and the genetic variability within a population, and is also used within the fields of forensic science, medical diagnosis, and animal and plant breeding.

Exam-style Questions

- 1 An agricultural company is creating a transformed wheat plant containing a gene for herbicide resistance. After announcing some early positive results, the company was approached by anti-genetic engineering activists. The company spoke with some of the activists to hear their concerns but continued production of the plant.
- 1.1 Suggest **two** ethical concerns that the anti-genetic engineering activists may have had with the agricultural company's work.

(2 marks)

Scientists at the company used a **DNA probe** to first locate the resistance gene.

- 1.2 What is a DNA probe?

(1 mark)

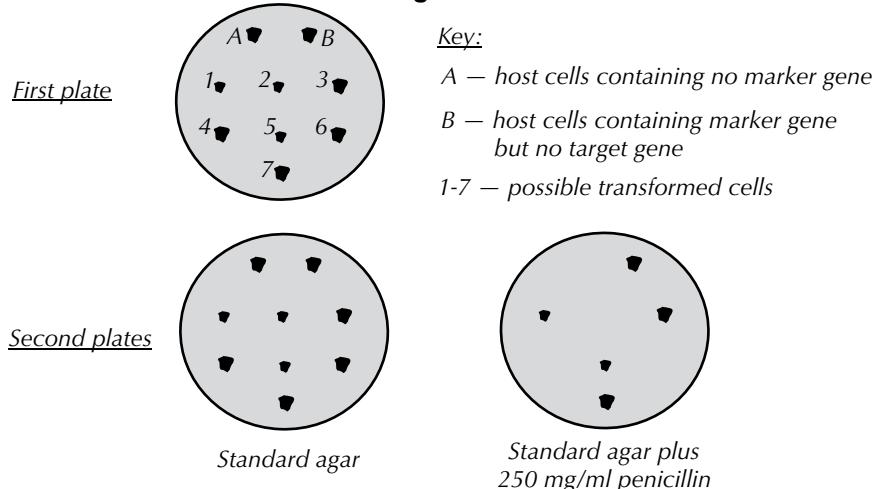
- 1.3 Describe how a DNA probe could have been used to locate the gene.

In your answer, you should make clear the sequence of the steps involved in locating the gene.

(4 marks)

The scientists used *in vivo* cloning techniques to introduce the gene into some host bacteria. The host bacteria were grown on standard agar plates to produce colonies and the colonies were then transferred to a second set of plates. The first and second sets of plates are shown in **Figure 1**.

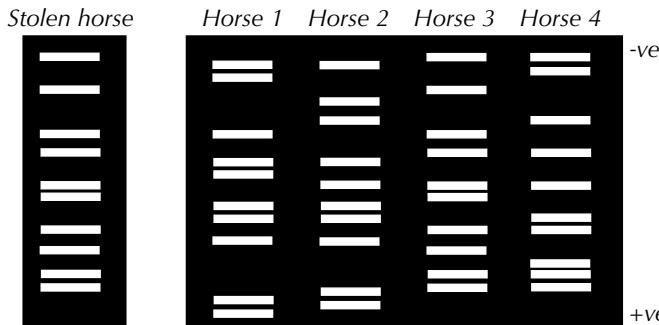
Figure 1



- 1.4 Explain why the colonies of bacteria were transferred to the second set of plates.
(3 marks)
- 1.5 Explain why the bacteria in **colony A** were added to the plates.
(1 mark)
- 1.6 Suggest **one** colony for use in further experiments on the transformed wheat plant. Explain your choice.
(1 mark)

- 2** A prize-winning race horse has been stolen from its stables. Police suspect it has been taken to a stud farm where it has previously gone to breed. The police have obtained DNA samples from four similar-looking horses at the stud farm and used them to produce genetic fingerprints to compare against a genetic fingerprint taken previously from the stolen animal. The genetic fingerprints are shown in **Figure 2**.

Figure 2



- 2.1** Describe and explain how the genetic fingerprints have been produced from the DNA samples. (5 marks)
- 2.2** Use your understanding of the biology behind genetic fingerprint technology to explain why the chances of two genetic fingerprints matching by chance are so small. (3 marks)
- 2.3** Is the stolen animal at the stud farm? Explain your answer. (1 mark)
- 2.4** Compare the genetic fingerprints of horse 4 and the stolen horse. Suggest a reason for these results. (3 marks)
- 2.5** Give **one** use for genetic fingerprint technology other than in forensic science. (1 mark)
- 3** Scientists are cloning the gene *BtrA* so they can study the effects of the protein it codes for in a species of fish. They start by using reverse transcriptase to obtain fragments of DNA containing the gene.
- 3.1** Describe **one** other method they could use to obtain a DNA fragment. (2 marks)
- The scientists next incubate the DNA fragments with a restriction enzyme to produce sticky ends, then use *in vivo* cloning techniques to introduce the gene into a bacterial cell along with a fluorescent marker gene.
- 3.2** Explain the importance of producing sticky ends for gene cloning. (1 mark)
- 3.3** Describe the *in vivo* cloning techniques used after the production of the sticky ends on the DNA fragments. (6 marks)



Exam Help

1. The Exams

You'll take three exams as part of A-level Biology. It seems obvious, but if you know exactly what will be covered in each of the exams, how much time you'll have to do them and how they'll be structured, you can be better prepared. So let's take a look at the ins and outs of the exams you'll be facing...

Exam Tip

All this exam info is only relevant if you're taking the A-level in Biology. If you're taking the AS-level, you'll be sitting a completely different set of papers, which are structured in a different way. There are two AS-level papers that both test Topics 1 to 4 (and Practical Skills).

Exam Tip

Even though you're taking an A-level in Biology, there will be some maths to do in these papers that's set in a biological context. There's lots more on the maths you could be tested on on pages 5 to 14, as well as in the Maths Skills examples throughout the book.

Exam Tip

Synoptic means you will need to draw together your knowledge of different areas of Biology in relation to a theme.

What's assessed in each paper?

AQA A-level Biology is examined in three papers taken at the end of the course. Papers 1 and 2 are each worth 35% of the total marks and Paper 3 is worth 30% of the total marks.

| Paper | Total marks | Time | Topics assessed |
|-------|-------------|---------|--|
| 1 | 91 | 2 hours | 1, 2, 3, 4 & relevant Practical Skills |
| 2 | 91 | 2 hours | 5, 6, 7, 8 & relevant Practical Skills |
| 3 | 78 | 2 hours | 1 to 8 & relevant Practical Skills |

All three A-level papers test you on Practical Skills — take a look at the Practical and Maths Skills section at the front of this book for more.

How are the exams structured?

- Papers 1 and 2 are mainly a mixture of short and long answer questions. Some of these questions will test you on the facts you need to know, some will test whether you can apply your knowledge to unfamiliar contexts and some will test your knowledge of Practical Skills. There'll even be a few calculation questions thrown in.
- Paper 1 also contains 15 marks' worth of extended response questions. These are questions that require you to write a longer answer with a logical structure. E.g. you could be asked to describe the steps in a particular process. These questions could involve an extended calculation too.
- Paper 2 also contains a 15 mark comprehension question. You'll be given a passage of information to read and will then need to answer the question parts that follow using both the information you've been given and your own scientific knowledge.
- Paper 3 is split into two sections. Section A has lots of questions on practical techniques and skills, with 15 marks being awarded for questions that ask you for a critical analysis of experimental data. For example, you could be given some data (e.g. in a graph or table) and asked to draw conclusions from it or you could be given a conclusion and asked to evaluate how well the data supports the conclusion. As for Papers 1 and 2, there'll also be fact recall questions, questions that test whether you can apply your knowledge, and calculation questions.
- Section B of Paper 3 consists of a 25 mark synoptic essay question. There's more on this on the next page.

Answering the essay question

You'll be given a choice of two essay titles in Section B of Paper 3 and asked to write about one of them. The titles are designed to get you to write about a range of material from both years of your A-level course. Writing an essay might seem like a daunting task, but don't panic. Here are some tips for getting top marks:

- Before you start your essay, it's a good idea to quickly scribble down a rough plan — this should help you to present your ideas in a clear, logical way. It should also stop you from repeating yourself or missing out any important bits. You should aim to write about at least five different topic areas.
- You'll need to clearly show how all the information you include is relevant to the essay title — don't just write down everything you know about a topic.
- The information you include must be detailed, scientifically correct and of A-level standard. 'Plants are green and have leaves' won't get you any marks at this level.
- You must use appropriate scientific terminology.
- Your essay should be well-written and clearly explained.
- To get the very highest marks, your answer should show evidence of wider reading (i.e. it should include things that aren't explicitly on the specification, but are still of a high standard and relevant to the question).

You'll get 2 hours in total for this paper and should aim to leave yourself about 50 minutes to plan and write your essay. This should be enough time to write about 3 sides of A4.

Exam Tip

Remember, you need to read the essay question carefully and answer the question you're asked — don't just rehash an old essay that you happen to have learnt off by heart.

Exam Tip

Making a quick plan of the topics you'll cover might help you decide which title you can write a better essay for.

Tip: There's an essay question for you to have a go at on the next page. It's worth timing yourself as you do it, so you get an idea of what it will be like to write the essay under exam conditions.

Solving problems in a practical context

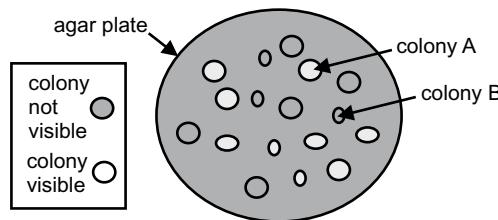
In the exams, you'll get plenty of questions set in a 'practical context'. As well as answering questions about the methods used or the conclusions drawn (see pages 1 to 18), you'll need to be able to apply your scientific knowledge to solve problems set in these contexts.

Example

- 1 A scientist amplified a gene by transferring a plasmid containing the target gene and a fluorescent marker gene into some bacterial cells. The cells were grown on an agar plate. The plate was then placed under UV light. The result is shown in Figure 1.

Figure 1

Agar plate under UV light



Exam Tip

Make sure you read all the information you're given at the start of an exam question carefully, and pay attention to what's being shown in any figures that are included too.

Exam Tip

Questions like this can look a bit scary, but you just have to apply what you already know about Biology to a real-life example. There are plenty of questions like this for you to have a go at in this book.

- 1.1 Which colony, **A** or **B**, contains transformed host cells? Explain your answer.

(2 marks)

You should remember from page 480 that the fluorescent marker gene is included in the plasmid so that bacterial cells that have taken up the plasmid (transformed cells) can be identified. Cells that contain the marker gene, and therefore the plasmid, will fluoresce under UV light. Cells that don't contain the marker gene won't be visible. So colony A contains transformed host cells and colony B does not.

Practice Question — Exam-style Question

- Q1 Write an essay about the importance of inorganic ions in living organisms.

(25 marks)

2. Command Words

Command words are just the bits of a question that tell you what to do.

Exam Tip

When you're reading exam questions, underline the command words. That way you'll know exactly what type of answer to give.

Exam Tip

If you're answering a longer 'compare' or 'evaluate' question make a mental list of the similarities and differences or pros and cons first, so you know what you want your answer to include before you start writing.

| Command word: | What to do: |
|---------------------|---|
| Give / Name / State | Give a brief one or two word answer, or a short sentence. |
| Describe | Write about what something's like, e.g. describe the structure of fish gills. |
| Explain | Give reasons for something. |
| Suggest | Use your scientific knowledge to work out what the answer might be. |
| Compare | Give the similarities and differences between two things. |
| Contrast | Give the differences between two things. |
| Calculate | Work out the solution to a mathematical problem. |
| Evaluate | Give the arguments both for and against an issue, or the advantages and disadvantages of something. You also need to give an overall judgement. |

Some questions will also ask you to answer ‘using the information/data provided’ (e.g. a graph, table or passage of text) or ‘with reference to figure X’ — if so, you must refer to the information, data or figure you’ve been given or you won’t get the marks. Make sure you quote the unit with any values taken from a graph or table too — the number alone is not enough.

Some questions may also ask you to answer ‘using your calculation’ — it’s the same here, you need to use your answer to a particular calculation, otherwise you won’t get the marks.

Not all of the questions will have command words — instead they may just ask a which / what / how type of question.

Exam Tip

Make sure you take a calculator (to help you with the calculation questions) and a ruler (with millimetre measurements) into all three of your exams. A pencil and a spare black pen may come in handy as well.

3. Time Management

Time management is really important in your exams — it’s no good writing a perfect answer to a 3 mark question if it takes you an hour.

For Papers 1 and 2, you get just over a minute per mark in each paper. So, if you get stuck on a short question, sometimes it’s worth moving on to another one and then coming back to it if you have time. Bear in mind that you might want to spend a bit longer than a minute per mark on the extended response and comprehension questions. For Paper 3, it’s a similar story — you’ll want to spend longer per mark on the essay question than on the shorter questions, so make sure you leave enough time for this at the end.

If you’ve got any time left once you’ve finished the paper, hold off on celebrating and have a look back through the questions. You can use the time to go back to any questions you’ve skipped, check your answers to calculation questions and to make sure you haven’t accidentally missed any questions out.

Exam Tip

If the question is only worth 1 mark, don’t waste time writing more than you need to. Questions worth more marks require longer answers.

Answers

Topic 1

Topic 1A — Biological Molecules

1. Molecules of Life

Page 22 — Application Question

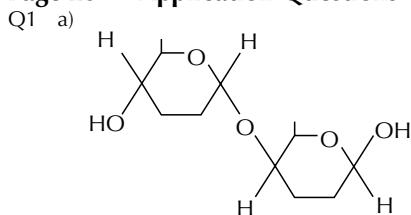
Q1 Cytochrome c is present in the cells of a wide variety of organisms, suggesting that they could all have descended from a common ancestor.

Page 22 — Fact Recall Questions

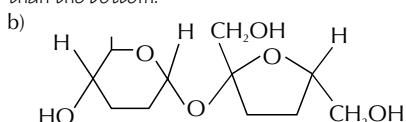
- Q1 A polymer is a large, complex molecule composed of many monomers joined together.
Q2 A monomer is a small, basic molecular unit that can form a polymer.
Q3 Any two from, e.g. monosaccharides / amino acids / nucleotides.
Q4 A chemical bond is formed between the monomers and a molecule of water is released.
Q5 A hydrolysis reaction.

2. Sugars

Page 25 — Application Questions



This diagram looks a bit different from other disaccharide diagrams. It's because the OH group needed to form the glycosidic bond is at the top of the galactose molecule rather than the bottom.



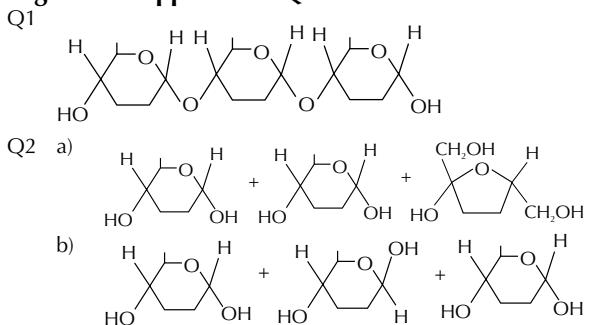
- Q2 Test 1 — no reducing sugars present, but non-reducing sugars might be present.
Test 2 — non-reducing sugars are present, but reducing sugars are not.
Test 3 — no sugars are present.
Test 4 — reducing sugars are present.
These tests are quite tricky. Think carefully about what sugars have been tested for and what the different colours of the results indicate. Remember that a negative result for a reducing sugar test doesn't rule out non-reducing sugars.

Page 25 — Fact Recall Questions

- Q1
- Q2 glycosidic
Q3 water
Q4 a) glucose and glucose
b) glucose and fructose
c) glucose and galactose
Q5 Add Benedict's reagent to a test sample and heat it in a water bath that's been brought to the boil. Look at the colour of the sample for the result. A positive result would be a coloured precipitate (green, orange, yellow or brick red, depending on the concentration of the reducing sugar) and a negative result would be blue.

3. Polysaccharides

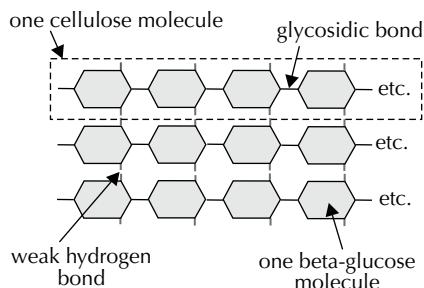
Page 26 — Application Questions



Page 28 — Fact Recall Questions

- Q1 monosaccharide
Q2 a) starch
b) glycogen
Q3 a) insoluble
b) It doesn't affect water potential so it doesn't cause water to enter cells by osmosis, which would make them swell.
Q4 a) A — amylopectin, B — amylose
b) It has lots of side branches, which means the enzymes that break amylopectin down can get to the glycosidic bonds easily. This means glucose can be released quickly when it is needed.
Q5 a) cellulose
b) Cellulose is made from long, unbranched chains of beta-glucose. These are joined by hydrogen bonds to form microfibrils. Microfibrils are very strong, which means they provide support/strength/rigidity in a cell wall.

Q6

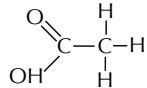


- Q7 Use the iodine test — add iodine dissolved in potassium iodide solution to a test sample. Look at the colour of the sample for the result. A positive result would be dark blue-black and a negative result would be a brownish-orange colour.

4. Lipids

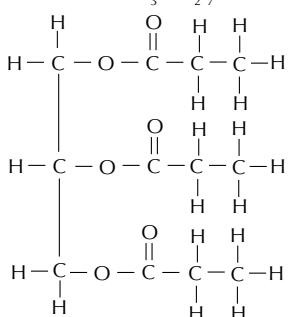
Pages 31–32 — Application Questions

Q1



- Q2 a) propanoic acid = $\text{CH}_3\text{CH}_2\text{COOH}$
 palmitic acid = $\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
 stearic acid = $\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$
 oleic acid = $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$

b)



c) oleic acid

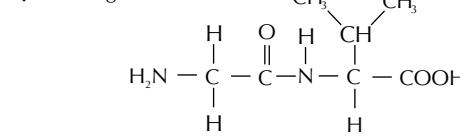
Page 32 — Fact Recall Questions

- Q1 A molecule of glycerol and three fatty acids.
- Q2 A saturated fatty acid doesn't have any double bonds between its carbon atoms, an unsaturated fatty acid does.
- Q3 A — phosphate group, B — glycerol, C — ester bond, D — fatty acid/hydrocarbon tail
- Q4 Because they contain lots of chemical energy and they're insoluble in water.
- Q5 Phospholipid heads are hydrophilic and their tails are hydrophobic, so they form a double layer with their heads facing out towards the water on either side. This makes the centre of the membrane bilayer hydrophobic, so water-soluble substances can't easily pass through it.
- Q6 a) lipids / fats / oils
 b) The student should shake the test substance with ethanol for about a minute, before pouring the solution into water. A milky emulsion will appear if the result is positive.

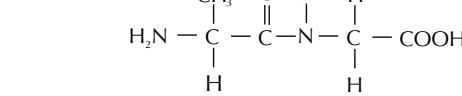
5. Proteins

Page 34 — Application Questions

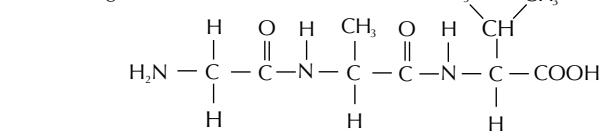
Q1 a)



b)



c)



Page 36 — Application Questions

Q1 Orange juice and goat's milk.

Q2 As a control.

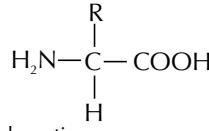
- Q3 a) The liquid needs to be alkaline for the test to work.
 b) Not added any/enough sodium hydroxide solution.

Page 36 — Fact Recall Questions

Q1 Amino acids

Q2 A chain of more than two amino acids joined together.

Q3



Q4 Condensation

Q5 Peptide

Q6 Hydrogen bonds, ionic bonds and disulfide bridges.

Q7 Structural proteins are made of long polypeptide chains lying parallel to each other with cross links between them. This makes them physically strong.

- Q8 a) sodium hydroxide solution
 b) copper(II) sulfate solution
 c) It would be purple.

6. Enzymes

Page 39 — Fact Recall Questions

Q1 extracellular

Q2 a)

b) The activation energy needed for the reaction with the presence of an enzyme.

Q3 Activation energy is needed to start a chemical reaction. The activation energy is often provided as heat. With the presence of an enzyme, the activation energy required to start a reaction is lowered. Therefore not as much heat is needed, so the reaction can take place at lower temperatures than it could do without an enzyme.

Q4 In the lock and key model the active site has a fixed shape that is complementary to the substrate, but in the induced fit model the active site has to change shape slightly to allow the substrate to bind tightly.

Q5 The enzyme's tertiary structure.

Q6 An enzyme can only bind with a substrate that has a complementary shape to its active site.

7. Factors Affecting Enzyme Activity

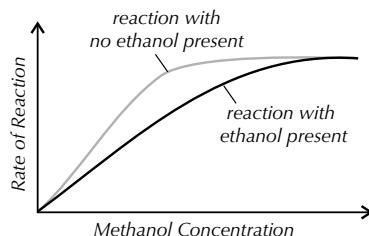
Page 43 — Application Questions

- Q1 a) i) C — the enzyme is still active at 80 °C. This means the bacteria can live at very high temperatures and therefore is hyperthermophilic.
ii) A — the enzyme is active at temperatures between 0 and 17 °C. This means the bacteria can live at very cold temperatures, so is psychrotrophic.
- b) A — The enzyme would become denatured at temperatures over 17 °C, so the enzyme activity would be reduced to zero.
B — There would be some enzyme activity but the rate of reaction would gradually decrease until temperatures of around 70 °C were reached. At this point the enzyme would be denatured and there would be no further enzyme activity at higher temperatures.
C — There would be an increasing amount of enzyme activity. The rate of reaction would gradually increase as the temperature increased.
- Q2 a) A — The rate of reaction is higher in relation to the hydrogen peroxide concentration. This is because there are more catalase molecules present, which means the hydrogen peroxide molecules will collide more frequently with the active sites.
b) The curves flatten out at the saturation point. All the active sites are full, so increasing the hydrogen peroxide concentration won't increase the rate of reaction any further.
- Q3 The initial rate of reaction is fastest at pH 5. The graph eventually reaches a plateau at pH 5 because all the substrate is used up. At pH 3, the enzyme's tertiary structure is disrupted and the shape of its active site is altered, so the reaction is slower and the graph doesn't reach a plateau because it takes longer for all the substrate to be used up.

Page 45 — Application Questions

- Q1 Ethanol has a similar shape to methanol. This means it will act as a competitive inhibitor, binding to the active site of alcohol dehydrogenase and blocking methanol molecules. This means lower levels of methanol will be hydrolysed so the toxic product (formaldehyde) won't build up to fatal levels.

Q2



Your curve should be lower than the rate of reaction without any ethanol present. The reaction won't stop completely as some of the methanol molecules will still bind with the active sites. The plateau should be later as the reaction won't reach its maximum rate until the methanol concentration is much higher. The curve should start at zero.

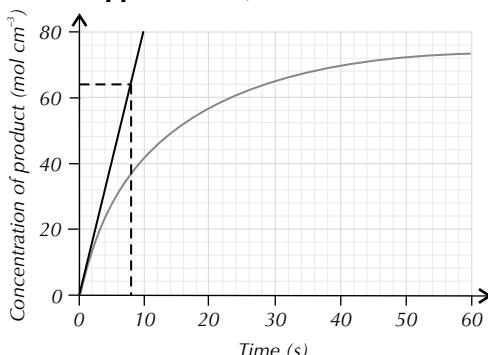
Page 45 — Fact Recall Questions

- Q1 At higher temperatures the molecules have more kinetic energy, so they move faster. This makes the substrate molecules more likely to collide with the enzymes' active sites. The energy of these collisions also increases, which means each collision is more likely to result in a reaction.
- Q2 A very high temperature makes the enzyme's molecules vibrate more. This vibration breaks some of the bonds/hydrogen bonds and ionic bonds that hold the enzyme in shape. The active site changes shape and the enzyme and substrate no longer fit together. The enzyme is denatured.
- Q3 The bonds that hold the enzyme in shape are broken. This alters the shape of the active site meaning it is no longer a complementary shape to the substrate. The enzyme can't catalyse the reaction.
- Q4 e.g. pH
- Q5 The point at which all active sites are occupied by substrate molecules.
- Q6 The rate of reaction stays constant. All active sites are occupied so increasing the substrate concentration has no effect.
- Q7 At first, increasing the enzyme concentration increases the rate of the reaction. This is because the more enzyme molecules there are in a solution, the more likely a substrate molecule is to collide with an active site and form an enzyme-substrate complex. The rate of reaction continues to increase until the substrate concentration becomes a limiting factor. At this point the rate of the reaction levels off.
- Q8 a) Away from the active site.
b) At the active site.
- Q9 A non-competitive inhibitor molecule binds to the enzyme away from the active site. Its presence alters the shape of the active site meaning that substrate molecules can no longer bind here. This prevents enzyme activity.

8. Enzyme-Controlled Reactions

Page 49 — Application Questions

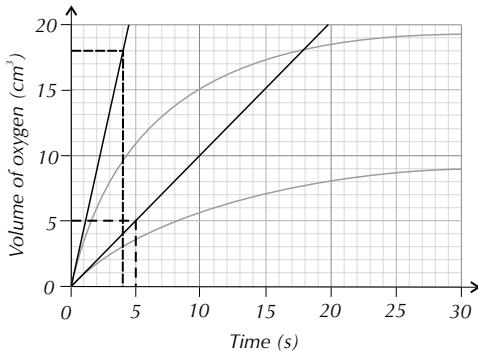
Q1



change in $y \div$ change in $x = 64 \text{ mol cm}^{-3} \div 8 \text{ s}$
 $= 8 \text{ mol cm}^{-3} \text{s}^{-1}$
 (accept answers between $6.5 \text{ mol cm}^{-3} \text{s}^{-1}$ and $10 \text{ mol cm}^{-3} \text{s}^{-1}$)
 Tangents are tricky things to draw — there'll usually be a small range of acceptable answers that will get the mark.

Q2 a) Any two from: e.g. temperature, pH, enzyme concentration

b)



2 mol dm^{-3} — change in $y \div$ change in $x = 18 \text{ cm}^3 \div 4 \text{ s}$
 $= 4.5 \text{ cm}^3 \text{s}^{-1}$
 (accept answers between $3.3 \text{ cm}^3 \text{s}^{-1}$ and $5 \text{ cm}^3 \text{s}^{-1}$)
 1 mol dm^{-3} — change in $y \div$ change in $x = 5 \text{ cm}^3 \div 5 \text{ s}$
 $= 1 \text{ cm}^3 \text{s}^{-1}$
 (accept answers between $0.8 \text{ cm}^3 \text{s}^{-1}$ and $1.3 \text{ cm}^3 \text{s}^{-1}$)

So $2 \text{ mol dm}^{-3} : 1 \text{ mol dm}^{-3} = 4.5 : 1$

Your answer depends on the values calculated for the tangents — it should fall between $6.25 : 1$ and $2.5 : 1$. See page 7 for help on calculating ratios.

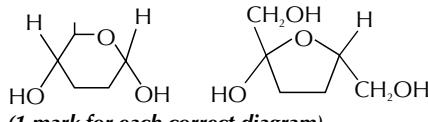
Page 49 — Fact Recall Question

Q1 E.g. set up boiling tubes containing the same volume and concentration of hydrogen peroxide. To keep the pH constant, add equal volumes of a suitable buffer solution to each boiling tube. Fill a measuring cylinder with water, turn it upside down and place it in a trough of water. Feed a delivery tube attached to a bung into the measuring cylinder. Put each boiling tube in a water bath set to a different temperature (e.g. 10°C , 20°C , 30°C and 40°C) along with another tube containing catalase. Wait 5 minutes before moving onto the next step so the enzyme gets up to temperature. Use a pipette to add the same volume and concentration of catalase to each boiling tube. Then quickly attach the bung and delivery tube. Record how much oxygen is produced in the first minute (60 s) of the reaction. Use a stopwatch to measure the time. Repeat the experiment at each temperature three times, and use the results to find the mean volume of oxygen produced. Calculate the mean rate of reaction at each temperature by dividing the volume of oxygen produced by the time taken (i.e. 60 s).

Exam-style Questions — pages 51-52

1.1 Sucrose is made from a fructose molecule (**1 mark**) and a glucose molecule (**1 mark**) which are joined by a glycosidic bond (**1 mark**) formed during a condensation reaction (**1 mark**).

1.2



(**1 mark for each correct diagram**).

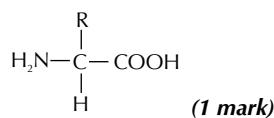
You don't need to learn the structure of fructose, but you do need to know that glucose and fructose are the monomers of sucrose.

1.3 Initially the sample is heated in a water bath (that's been brought to the boil) with Benedict's reagent to rule out the presence of reducing sugars (**1 mark**). A new test sample is then heated in a water bath (that's been brought to the boil) with dilute hydrochloric acid (**1 mark**) and then neutralised with sodium hydrogencarbonate (**1 mark**). Next the sample is heated with Benedict's reagent (**1 mark**). The test sample would form a coloured (green/yellow/orange/brick red) precipitate if a non-reducing sugar was present (**1 mark**).

1.4 Glycogen is a long, branched chain of α -glucose (**1 mark**). Animals use it to store excess glucose (**1 mark**). Many branches means that the glucose can be released quickly for energy (**1 mark**). It is also a compact shape, so it is easy to store (**1 mark**).

2.1 Add a few drops of sodium hydroxide solution to the test sample (**1 mark**). Then add some copper(II) sulfate solution (**1 mark**). If protein is present, the solution will turn purple (**1 mark**). If there's no protein present, the solution will stay blue (**1 mark**).

2.2 amino acid (**1 mark**)



2.3 The protein binds to pepsin's active site to form an enzyme-substrate complex (**1 mark**). This lowers the activation energy for the breakdown of the protein (by putting strain on the peptide bonds, making them easier to break) (**1 mark**). The reaction is catalysed and the products are released (**1 mark**).

- 2.4 The tertiary structure is the 3D structure of the polypeptide chain (**1 mark**), formed by hydrogen bonding and ionic bonding between different parts of the chain (**1 mark**). Disulfide bridges can also be formed between cysteine R groups (**1 mark**). The tertiary structure determines the shape of the active site of an enzyme (**1 mark**). The shape of the active site makes the enzyme specific to its substrate (**1 mark**).
- 3.1 (**1 mark for a value between pH 4 and pH 5**)
- 3.2 pH 1 and pH 9 (**1 mark**). There is no reaction at these pH levels (**1 mark**).
- 3.3 The shape of the active site has changed (**1 mark**) so it is no longer complementary in shape to the substrate, and will not bind to it to catalyse the reaction (**1 mark**).
- 3.4 Any two from: e.g. temperature, substrate concentration, enzyme concentration (**1 mark for each correct variable**).
- 3.5 A. The rate at which diglycerides and fatty acids are produced/the reaction rate is higher without the presence of orlistat (**1 mark**).
- 3.6 Molecules of orlistat have a similar shape to triglycerides (**1 mark**). They bind to the active sites of gastric lipase and block the entry of triglycerides (**1 mark**). This means the reaction that produces diglycerides and fatty acids can't take place as quickly (**1 mark**).

Topic 1B — More Biological Molecules

1. DNA and RNA

Page 55 — Application Questions

- Q1 a) TGACAGCATCAGCTACGAT
b) ACGTGGTACACCATTAGC

- Q2 a) 22
b) 12
c) 12

If there are 34 base pairs in total and 22 of them contain adenine, then the other 12 must contain both cytosine and guanine — it's all to do with complementary base pairing.

Page 56 — Fact Recall Questions

- Q1 It stores genetic information.
- Q2 RNA and proteins.
- Q3 Nucleotides
- Q4 A = phosphate group, B = pentose/pentose sugar,
C = nitrogen-containing organic base
- Q5 A DNA nucleotide contains a phosphate group, the pentose sugar deoxyribose and a nitrogen-containing organic base.
- Q6 adenine, guanine, cytosine and thymine
- Q7 Phosphodiester bonds
- Q8 Two DNA polynucleotide strands join together by hydrogen bonding between complementary base pairs — A with T and G with C. Two hydrogen bonds form between A and T, and three hydrogen bonds form between C and G. The antiparallel strands then twist round each other to form the DNA double helix.
- Q9 ribose
- Q10 adenine, guanine, cytosine and uracil
- Q11 Any three from: e.g. DNA is double-stranded, whereas RNA is single-stranded / the pentose sugar in DNA is deoxyribose but it's ribose in RNA / DNA contains the base thymine, whereas RNA doesn't (it contains uracil instead) / molecules of RNA are relatively short compared to longer DNA molecules.
- Q12 DNA has a relatively simple chemical composition.

2. DNA Replication

Page 58 — Application Questions

Q1



In semi-conservative replication of DNA, each of the new molecules of DNA contains a strand from the original molecule (shown in black in the diagram) and a new strand (grey in the diagram).

- Q2 AGTACCATGGATT

Page 60 — Application Question

- Q1 a) DNA in the two tubes were taken from bacteria that were grown in nutrient broths containing nitrogen isotopes of different weights. Tube A contained DNA with ¹⁴N (light nitrogen) incorporated, whereas tube B contained DNA with ¹⁵N (heavy nitrogen) incorporated. This meant that the sample in tube B settled further down the tube after being centrifuged because it was heavier.
- b) The DNA sample settled out in the middle of where the ¹⁴N (light nitrogen) DNA settled out and where the ¹⁵N (heavy nitrogen) DNA settled out. This is because the new bacterial DNA molecules contained one strand of the old DNA containing ¹⁵N (heavy nitrogen) and one strand of new DNA containing ¹⁴N (light nitrogen), as the new bacterial DNA had replicated semi-conservatively in the ¹⁴N (light nitrogen).
- c) Tube D contains two bands. The lower band is at the same position as the band in tube C and the upper band is at the same position as the band in tube A. This is because the DNA molecules from tube C, which contained one strand of the ¹⁵N (heavy nitrogen) DNA and one strand of the ¹⁴N (light nitrogen) DNA had replicated for another generation in ¹⁴N (light nitrogen). This meant that some of the molecules had ¹⁵N DNA template strands with ¹⁴N nucleotides incorporated onto new strands (these formed the lower band), and others had ¹⁴N DNA template strands with ¹⁴N nucleotides incorporated onto new strands (these formed the upper band).

Page 60 — Fact Recall Questions

- Q1 It's where half of the new strands of DNA are from the original molecule of DNA.
- Q2 DNA helicase, DNA polymerase
- Q3 DNA helicase breaks the hydrogen bonds between bases on the two polynucleotide DNA strands. This makes the helix unwind to form two single strands.
- Q4 Free-floating DNA nucleotides are attracted to their complementary exposed bases on each original template strand — A with T and C with G.

3. ATP

Page 62 — Application Questions

- Q1 AMP is made from a molecule of adenine, a molecule of ribose and one phosphate group.
- Q2 The breakdown/hydrolysis of ATP releases energy, so when this reaction/hydrolysis is coupled to the process of active transport it provides the energy for this process directly.

Page 62 — Fact Recall Questions

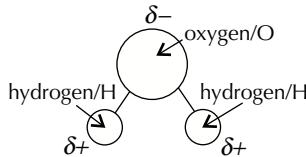
- Q1 Adenosine triphosphate
- Q2 A molecule of ATP is made from a molecule of adenine, a molecule of ribose and three phosphate groups.
- Q3 a) ADP and P_i
b) hydrolysis
c) ATP hydrolase
- Q4 a) It can be used to phosphorylate another compound/ added to another compound, which often makes the compound more reactive.
b) P_i
- Q5 a) condensation
b) E.g. photosynthesis / respiration

4. Water

Page 65 — Fact Recall Questions

- Q1 E.g. condensation and hydrolysis.
- Q2 Because it has a slight negative charge on one side and a slight positive charge on the other.

Q3



- Q4 A weak bond between a slightly positively charged hydrogen atom in one molecule and a slightly negatively charged atom in another molecule.
- Q5 A substance involved in a metabolic reaction.
- Q6 Its polarity/the fact that it is a polar molecule.
- Q7 Lots of heat is used to change it from a liquid to a gas.
- Q8 Because when water is heated, a lot of the heat energy is used to break the hydrogen bonds between water molecules. This means there is less heat energy available to actually increase the temperature of the water.
- Q9 a) The attraction between molecules of the same type (e.g. two water molecules).
b) Strong cohesion between water molecules allows water to travel in columns in the xylem tissue inside plants. Substances are transported around plants in this way.

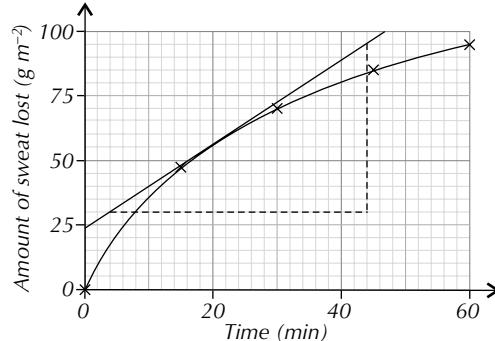
5. Inorganic Ions

Page 67 — Fact Recall Questions

- Q1 An ion that doesn't contain carbon.
- Q2 They are present (in solution) in the cytoplasm of cells and in the body fluids of organisms.
- Q3 Its specific properties.
- Q4 a) Iron ions.
b) They bind to oxygen.
- Q5 Hydrogen ions/H⁺
- Q6 a) Sodium ions/Na⁺
b) Co-transport/co-transportation

Exam-style Questions — pages 68-69

- 1.1 ATP also contains a ribose molecule (**1 mark**) and a molecule of adenosine (**1 mark**).
- 1.2 ATP synthase (**1 mark**)
- 1.3 E.g. ADP / AMP / a DNA nucleotide / a RNA nucleotide (**1 mark**).
- 1.4 During ATP hydrolysis ATP is broken down into a molecule of ADP and a phosphate group/P_i (**1 mark**). A phosphate bond is broken and this releases energy (**1 mark**). This reaction is catalysed by ATP hydrolase (**1 mark**).
- 1.5 ATP hydrolysis can be coupled to reactions that require energy so that energy can be supplied directly to the reaction and allow it to take place (**1 mark**). DNA helicase must therefore require energy in order to unwind DNA molecules by breaking hydrogen bonds (**1 mark**).
- 2.1 Water has a high latent heat of vaporisation (**1 mark**), which means it carries away a lot of heat energy when it evaporates (vaporises) from a surface (**1 mark**). So when sweat evaporates from the body it carries away heat energy, which cools the surface and helps to lower the temperature of the body (**1 mark**).
- 2.2 Water is a polar molecule/has a slightly positively charged end and a slightly negatively charged end (**1 mark**). The slightly negatively charged end of a water molecule will be attracted towards these positively charged sodium ions (**1 mark**). The ions will become totally surrounded by water molecules and this means they are dissolved in water (**1 mark**).
- 2.3 E.g. a molecule of glucose / an amino acid can be co-transported into a cell alongside sodium ions (**1 mark**).
- 2.4 45 g m⁻² (**1 mark**)
- 2.5



$$65 \div 40 = 1.6 \text{ g m}^{-2} \text{ min}^{-1}$$

(1 mark for the correct answer and 1 mark for correct units)

- (Accept answers between $1.5 \text{ g m}^{-2} \text{ min}^{-1}$ and $1.8 \text{ g m}^{-2} \text{ min}^{-1}$.)
- 3.1 B, C, A, D (**1 mark**)
- 3.2 DNA polymerase (**1 mark**)
- 3.3 A condensation reaction (**1 mark**).
- 3.4 It's where the base adenine (A) always pairs with thymine (T) and guanine (G) always pairs with cytosine (C) (**1 mark**). Two hydrogen bonds form between A and T, and three hydrogen bonds form between C and G (**1 mark**).
- 4.1 DNA stores genetic information while RNA transfers genetic information from the DNA to ribosomes (**1 mark**).
- 4.2 In a DNA nucleotide part A is deoxyribose, whereas in an RNA nucleotide it is ribose (**1 mark**).
- 4.3 Any two from: DNA is double stranded whereas RNA is single stranded (**1 mark**) / DNA contains thymine/T but this is replaced by uracil/U in RNA (**1 mark**) / RNA molecules are relatively short whereas DNA molecules are quite long (**1 mark**).

Topic 2

Topic 2A — Cell Structure and Division

1. Eukaryotic Cells and Organelles

Page 76 — Application Questions

- Q1 A = crista, B = outer membrane, C = matrix
Q2 Golgi apparatus
Q3 a) Mitochondria, to provide lots of energy for muscle contraction.
b) E.g. they might have a lot of lysosomes to enable them to break down invading pathogens.
c) E.g. ribosomes, rough endoplasmic reticulum, Golgi apparatus.

You need to specify the rough endoplasmic reticulum in this answer (as the smooth endoplasmic reticulum is involved in lipid synthesis, not protein synthesis).

- d) E.g. They might have microvilli on their surface to increase the surface area for reabsorbing molecules.

Page 76 — Fact Recall Questions

- Q1 To regulate movement of substances into and out of the cell.
To respond to chemicals like hormones.
Q2 A = nuclear envelope, B = nucleolus, C = chromatin, D = nuclear pore.
Q3 The nucleus controls the cell's activities by controlling the transcription of DNA.
Q4 It is a group of fluid-filled membrane-bound flattened sacs.
Q5 Any one from: To digest invading cells. / To break down worn out components of the cell.
Q6 It synthesises and processes lipids.
Q7 A tissue is where similar cells are grouped together, whereas an organ is where different tissues are grouped together to perform a particular function.
Q8 An organ system is where different organs work together to carry out a particular function.

2. Prokaryotic Cells and Viruses

Page 79 — Application Questions

- Q1 A = cell wall, B = capsule, C = plasmid, D = flagellum
Q2 Feature B (capsule) is a layer of slime that can help protect the bacterium from attack by the host's immune system.
Feature D (flagellum) allows the bacteria to move through the gut.
Q3 The main genetic material floats free in the cytoplasm. It is one long coiled-up strand of circular DNA, which is not attached to any histones. Other genetic material is in the form of small loops of DNA called plasmids. These contain genes for things like antibiotic resistance.

Page 79 — Fact Recall Questions

- Q1 In the cell wall.
Q2 Binary fission. The circular DNA and plasmids replicate. The main DNA loop is only replicated once but the plasmids can be replicated several times. The cell gets bigger and the DNA loops move to opposite poles of the cell. The cytoplasm begins to divide and new cell walls begin to form. The cytoplasm divides and two daughter cells are produced. Each daughter cell has one copy of the circular DNA but a variable number of copies of the plasmids.
Q3 The protein coat that surrounds the core of genetic material in a virus.
Q4 They allow a virus to attach to specific host cells, which have the complementary receptor proteins.

3. Analysis of Cell Components

Page 81 — Application Questions

- Q1 a) size of image ÷ magnification = size of real object
 $8 \text{ mm} \div 3150 = 0.0025 \text{ mm}$
b) size of image ÷ magnification = size of real object
 $18 \text{ mm} \div 3150 = 0.0057 \text{ mm}$
Q2 size of real object × magnification = size of image
 $0.00002 \text{ mm} \times 40 = 0.0008 \text{ mm} = 8 \times 10^{-4} \text{ mm}$
Q3 size of image ÷ magnification = size of real object
 $13 \text{ mm} \div 7000 = 0.0019 \text{ mm}$
Then times by 1000 to convert to μm
 $0.0019 \text{ mm} \times 1000 = 1.9 \mu\text{m}$
Q4 size of real object × magnification = size of image
 $0.023 \mu\text{m} \times 1500 = 34.5 \mu\text{m}$
Then divide by 1000 to convert to mm
 $34.5 \mu\text{m} \div 1000 = 0.035 \text{ mm}$
Q5 a) size of image ÷ size of real object = magnification
 $16 \text{ mm} \div 2 \text{ mm} = \times 8$
b) size of real object × magnification = size of image
 $3 \text{ mm} \times 50 = 150 \text{ mm}$
Q6 First you need to convert $10 \mu\text{m}$ to millimetres by dividing by 1000:
 $10 \mu\text{m} \div 1000 = 0.01 \text{ mm}$
size of image ÷ size of real object = magnification
 $10 \text{ mm} \div 0.01 \text{ mm} = \times 1000$

Page 85 — Application Questions

- Q1 a) Optical microscope, as electron microscopes can only be used on dead specimens.
b) SEM, as they can give 3-D images.
c) Electron microscope (TEM/SEM) as the virus particles are smaller than the maximum resolution of optical microscopes.
Q2 a) nuclei, mitochondria, lysosomes, ER, ribosomes
This is the filtered solution, so it should contain all the organelles.
b) Nuclei
Nuclei are the heaviest, so will separate out first.
c) ER, ribosomes.
The supernatant in this tube should contain everything except the nuclei (separated out in the first spin), mitochondria (separated out in the second spin) and the lysosomes (in the pellet in the bottom of Tube D).

Page 85 — Fact Recall Questions

- Q1 magnification = size of image ÷ size of real object
- Q2 Magnification is how much bigger the image is than the specimen. Resolution is how well a microscope can distinguish between two points that are close together.
- Q3 a) 0.2 µm
b) 0.0002 µm
- Q4 Electron microscope
- Q5 An electron microscope.
Lysosomes are too small to be seen with an optical microscope — they're less than 0.2 µm in diameter (the maximum resolution of an optical microscope).
- Q6 TEMs use electromagnets to focus a beam of electrons, which is then transmitted through the specimen. Denser parts of the specimen absorb more electrons, which makes them look darker on the image you end up with.
- Q7 SEMs scan a beam of electrons across the specimen. This knocks off electrons from the specimen, which are gathered in a cathode ray tube to form an image.
- Q8 Advantage: e.g. gives high resolution images, so can be used to look at small objects / the internal structure of organelles. Disadvantage: any one from, e.g. can only be used on thin specimens. / Can only be used on non-living specimens. / Produces black and white images. / Images may contain artefacts.
- Q9 Any one from, e.g. can be used on thick specimens, whereas TEMs can't. / Can produce 3-D images, whereas TEMs can't.
- Q10 A prepared microscope slide in which the specimen has been suspended in a drop of liquid.
- Q11 Something you can see down the microscope that isn't part of the cell or specimen you are looking at.
- Q12 They repeatedly prepared specimens in different ways. If an object could be seen with one preparation technique, but not another, it was more likely to be an artefact than an organelle.
- Q13 By vibrating the cells, or by grinding the cells up in a blender.
- Q14 The homogenised cell solution is filtered through a gauze to separate any large cell debris or tissue debris, like connective tissue, from the organelles.

4. Cell Division — Mitosis

Page 88 — Application Questions

- Q1 a) B — because the chromosomes have lined up down the middle of the cell and are attached to spindle fibres.
b) A — because the centromeres have divided, separating each pair of sister chromatids and the spindle fibres have contracted, pulling the chromatids to opposite poles of the spindle by their centromeres.
- To answer this you need to quickly go through each stage of mitosis in your head and think about the main thing that's happening, e.g. in metaphase all the chromosomes are in middle of the cell. Then ask yourself if you can see that in the photo.
- Q2 a) 12-16 hours and 36-40 hours, because the mass of DNA doubles.
b) 24 hours and 48 hours, because the mass of DNA halves.
c) i) Two (at 24 and 48 hours) because the mass of the cell and its DNA doubles and halves twice.
ii) At 72 hours.
- Q3 12 out of 150 cells are in prophase. This suggests that the proportion of time the cells spend in prophase is 12/150th of the cell cycle. The cell cycle lasts 0.70 days, so $0.70 \times 24 \text{ hours} = 16.8 \text{ hours}$.
The cells spend $(12 \div 150) \times 16.8 = 1.3 \text{ hours in prophase}$.

Page 89 — Application Question

- Q1 a) synthesis / interphase
b) mitosis
Methotrexate stops A and G nucleotides from forming — these nucleotides are needed to make new strands of DNA during DNA synthesis. Spindle fibres separate chromosomes during mitosis.

Page 89 — Fact Recall Questions

- Q1 The process that all body cells from multicellular organisms use to grow and divide.
- Q2 For growth and for repairing damaged tissues.
- Q3 Interphase
- Q4 During prophase the chromosomes condense, getting shorter and fatter. The centrioles start moving to opposite ends of the cell, forming the spindle. The nuclear envelope breaks down and chromosomes lie free in the cytoplasm.
- Q5 During telophase the chromatids reach the opposite poles on the spindle. They uncoil and become long and thin again. They're now called chromosomes again. A nuclear envelope forms around each group of chromosomes, so there are now two nuclei. The cytoplasm finishes dividing and there are now two daughter cells that are genetically identical to the original cell and to each other.
- Q6 Division of the cell cytoplasm.
- Q7 It's a tumour that invades surrounding tissues.

5. Investigating Mitosis

Page 93 — Application Questions

- Q1 a) To make the chromosomes easier to see under the microscope.
b) E.g. toluidine blue O / ethano-orcein / Feulgen stain
c) The student should have put a coverslip over the top of the specimen and pushed down firmly, making sure that he didn't smear the coverslip sideways.
d) The mitotic index for the root tip cells would be higher because they are part of a tissue that is undergoing a lot of growth — unlike the mature leaf.
- Q2 a) $10 \div 6.5 = 1.5 \mu\text{m}$
b) $14 \times 1.5 = 21 \mu\text{m}$
- Q3 a) $(207 \div 750) \times 100 = 27.6\%$
b) The tissue may be undergoing repair or cancerous growth may be occurring.
c) $(9.0 \div 200) = 0.045 \text{ mm}$

Page 93 — Fact Recall Questions

- Q1 The slide containing the specimen should first be clipped onto the slide. Then an objective lens should be selected and the coarse adjustment knob used to position the objective lens just above the slide. Finally, while looking down the eyepiece, the fine adjustment knob should be used to adjust the focus until a clear image of the specimen can be seen.
- Q2 An eyepiece graticule is fitted onto the eyepiece. It's like a transparent ruler with numbers, but no units.
- Q3 A stage micrometer is used to work out the value of the divisions on the eyepiece graticule at a particular magnification.

Exam-style Questions — pages 95-96

- 1.1 No. The microscope has a resolution of $200 \text{ nm}/0.2 \mu\text{m}$, which means it can't distinguish between objects that are smaller than $200 \text{ nm}/0.2 \mu\text{m}$ — such as the ribosomes (**1 mark**). If you convert the diameter of the ribosomes and the resolution of the microscope into the same units, (e.g. both nm or both μm) it's easier to see that the ribosomes are too small for the microscope to pick up.
- 1.2 size of real object = size of image \div magnification
 $= 4 \div 100 = 0.04 \text{ mm}/40 \mu\text{m}$ (**1 mark**)
- 1.3 Any one from, e.g. ribosomes/rough endoplasmic reticulum as these are the site of protein synthesis. / Golgi apparatus because this processes and packages new proteins.
(**1 mark for sensible choice of organelle, 1 mark for correct explanation**)
- 1.4 Any five from: e.g. First the cell membranes are broken up by homogenisation to release the organelles into solution (**1 mark**). The solution is kept ice cold to prevent enzymes breaking down the organelles / an isotonic solution is used to prevent damage to organelles by osmosis / a buffer solution is added to maintain the pH (**1 mark**). The homogenised cell solution is then filtered through a gauze to separate any large cell debris or tissue debris from the organelles (**1 mark**). Ultracentrifugation is then carried out to separate each organelle from the others (**1 mark**). The cell fragments are poured into a tube and spun in a centrifuge to separate out the heaviest organelle, which remains in the pellet at the bottom of the tube, leaving the others suspended in the supernatant (**1 mark**). This process is then repeated at higher and higher speeds to separate out all the organelles (**1 mark**).
(**Maximum of 5 marks available**)
- 2.1 Bacteria are prokaryotic cells, so the penicillin inhibits the synthesis of their cell walls, eventually leading to cell lysis and death (**1 mark**). Human cells are eukaryotic animal cells, and so have no cell wall, so penicillin antibiotics leave these cells unaffected (**1 mark**).
- 2.2 E.g. Antibiotics could target the capsule of prokaryotic cells, which human cells don't have (**1 mark**). This would leave the prokaryotes more open to attack from the cells of the host's immune system (**1 mark**). The question asks for an example. Marks could be awarded for any example of a bacterial feature that human cells don't have and a sensible explanation of why it would be appropriate.
- 3.1 A is gap phase 1 because it contains 1 arbitrary unit of DNA (**1 mark**). B is synthesis because the mass of DNA is increasing (**1 mark**). C is gap phase 2 because it contains 2 arbitrary units of DNA (**1 mark**). The key to this question is to look at the amount of DNA at each stage on the graph and link that back to how the amount of DNA in a cell changes during the phases of interphase.
- 3.2 E.g. because phase A lasts longer (**1 mark**). If each phase lasted the same length of time, then each phase would have broadly the same number of cells.
- 4.1 The data shows a positive correlation between the activity of protein X and the percentage of cells dividing (**1 mark**), but you can't tell from the data that the activity of protein X is causing the cells to divide — it might just be a coincidence / there might be other factors involved (**1 mark**). Also the graph only shows data for one species of yeast so you can't apply any trend to other species of yeast (**1 mark**).
- 4.2 E.g. cell division could be measured in yeast cells that do not produce this protein (**1 mark**). The purpose of this would be to make sure that the change in the percentage of cells dividing is due to the activity of protein X and nothing else (**1 mark**).

- 5.1 It is an organ because it is made up of lots of different tissues, such as the cornea and the retina (**1 mark**), which work together to allow us to see (**1 mark**).
- 5.2 A mutation in the gene for Rb means the Rb protein is not made / a faulty version of the Rb protein is made (**1 mark**). This means the damaged cell goes through the cell cycle and divides (**1 mark**). The cells continue to divide which can lead to the formation of a tumour (**1 mark**).

Topic 2B — Cell Membranes

1. Cell Membranes — The Basics

Page 99 — Application Questions

- Q1 a) E.g. to keep the enzymes needed for photosynthesis all in one place/to compartmentalise photosynthesis, making photosynthetic reactions more efficient.
- b) E.g. to control what substances enter and leave the cell. / To enable cell signalling.
- Q2 E.g. using carrier proteins/channel proteins in the membrane.
- Q3 E.g. energy-releasing organelles require lots of substances (e.g. nutrients, enzymes, ATP) to travel across their membranes. Some of these substances will require help from proteins to get across the membrane, so these membranes will have a higher protein content.
- Q4 Freezing the raspberries will have caused ice crystals to form and pierce the cell-surface membranes, making the membranes highly permeable when they thawed. This will have caused the red pigment to leak out of the raspberry cells as they defrosted.

Page 101 — Application Question

- Q1 a) E.g. the size of the beetroot cubes. / The beetroot the cubes came from. / The volume of methanol solution the cubes were soaked in. / The temperature of the equipment and surroundings.
- b) Any two from: e.g. it should be turned on and left for five minutes to stabilise. / It should be set up so it's using the correct (blue) filter / a wavelength of about 470 nm. / It should be calibrated to zero (using a cuvette containing distilled water).
- c) As the concentration of methanol increased, more of the lipids in the beetroot's cell membranes would dissolve. This would cause the cells to lose their structure and become more permeable. More pigment would be released from the beetroot cubes, so the absorbance of the surrounding liquid would increase.

Page 101 — Fact Recall Questions

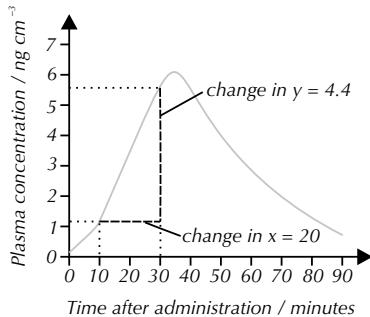
- Q1 A = glycoprotein, B = glycolipid, C = cholesterol, D = protein channel, E = phospholipid (head)
- Q2 Because the phospholipids are constantly moving.
- Q3 Some of the proteins are fixed in position, others move sideways.
- Q4 A protein with a carbohydrate attached.
- Q5 'Hydrophilic' means 'attracts water'. Hydrophobic means 'repels water'.
- Q6 The centre of the phospholipid bilayer is hydrophobic, so the membrane doesn't allow water-soluble substances through it.
- Q7 Some proteins in the membrane allow the passage of large or charged particles that would otherwise find it difficult to cross the membrane.
- Q8 Cholesterol helps make the membrane less fluid and more stable. It maintains shape of animal cells and creates a barrier to polar substances.
- Q9 E.g. cut equal sized beetroot cubes and place each cube in a test tube. Add the same volume of water to each test tube, then place each test tube in a water bath set at a different temperature and leave them for the same length of time. Remove the beetroot cubes from each test tube and then use a colorimeter to measure the absorbance of the remaining liquid. This will indicate how much pigment has been released by each beetroot cube, and therefore how permeable the membrane is at each temperature tested.

2. Diffusion

Page 105 — Application Questions

- Q1 The ink molecules are moving from an area of higher concentration (the original drop of ink) to an area of lower concentration (the surrounding water).
- Q2 a) The distance the particles have to travel is further so the rate of diffusion will decrease.
b) The surface area of the cell will increase, so the rate of diffusion will increase.
c) The concentration gradient will increase, so the rate of diffusion will increase.
- Q3 You could increase the concentration gradient of the particle and monitor the rate of diffusion. Facilitated diffusion requires proteins to transport particles across the cell membrane. There are a fixed number of proteins in the membrane. Once all the proteins are in use, increasing the concentration gradient won't increase the rate of facilitated diffusion any further, whereas increasing the concentration gradient will continue to increase the rate of simple diffusion.

Q4



$$\text{Rate} = \frac{4.4}{20} = 0.22 \text{ ng cm}^{-3} \text{ min}^{-1}$$

Your 'change in y' could have been anywhere between 4.2 and 4.6 giving you an answer between 0.21 and 0.23 $\text{ng cm}^{-3} \text{ min}^{-1}$.

Page 105 — Fact Recall Questions

- Q1 The net/passive movement of particles from an area of higher concentration to an area of lower concentration.
- Q2 It's a passive process.
- Q3 E.g. concentration gradient, surface area, thickness of the exchange surface
- Q4 It's a passive process.
- Q5 First, a large molecule attaches to a carrier protein in the membrane. Then, the protein changes shape. This releases the molecule on the opposite side of the membrane.
- Q6 Channel proteins are proteins within a cell membrane that form pores.
- Q7 Channel proteins allow charged particles to pass through a cell membrane via facilitated diffusion.
- Q8 It would increase the rate of facilitated diffusion as it would allow more particles to be transported across the membrane at the same time.

3. Osmosis

Page 109 — Application Questions

- Q1 a) Water molecules will move from the cheek cells into the salt solution.
A -300 kPa solution has a higher water potential (it's less negative) than a -325 kPa solution.
b) Water molecules will move into the apple slices out of the beaker of water.
c) There will be no net movement of water molecules as the water potential in both solutions is the same/the solutions are isotonic.
- Q2 a) The potato cells have a lower water potential than the sucrose solution, so they gain water by osmosis.
b) The cells in both solutions will decrease in volume. This is because they have a higher water potential than the sucrose solutions, so will lose water by osmosis.
- Q3 a) $1.5 \text{ M} = 1500 \text{ mM}$.
Scale factor = $1500 \text{ mM} \div 125 \text{ mM} = 12$.
 $30 \div 12 = 2.5$.
So she needs to use 2.5 cm^3 of the original solution and $30 - 2.5 = 27.5 \text{ cm}^3$ of distilled water.
b) She is starting with 30 cm^3 and diluting by a factor of 5. $30 \text{ cm}^3 \div 5 = 6 \text{ cm}^3$, so she would need to add $30 - 6 = 24 \text{ cm}^3$ of distilled water to two test tubes. She would then use a pipette to take 6 cm^3 from the 125 mM solution and add it to one of the test tubes containing 24 cm^3 of distilled water. She would then mix the contents of this test tube thoroughly (to create one solution) before taking 6 cm^3 of the solution and adding it to the distilled water in the remaining test tube and mixing thoroughly (to create the second solution).

Page 109 — Fact Recall Questions

- Q1 Osmosis is the diffusion of water molecules across a partially permeable membrane, from an area of higher water potential to an area of lower water potential.
- Q2 Water potential is the potential/likelihood of water molecules to diffuse out of or into a solution.
- Q3 E.g. The water potential gradient (the higher the water potential gradient, the faster the rate of osmosis). The thickness of the exchange surface (the thinner the exchange surface, the faster the rate of osmosis). The surface area of the exchange surface (the larger the surface area, the faster the rate of osmosis).
- Q4 E.g. cut equal sized chips from a potato. Divide the chips into groups of three and measure the mass of each group. Make up several different sucrose concentrations. Place each group of chips into a different sucrose solution and leave all the chips for the same length of time. Remove the chips and measure the mass of each group again. Record each group's percentage change in mass, then make a calibration curve by plotting the percentage change in mass against the concentration of the sucrose solution the group was in. Read off the concentration where the curve crosses the x-axis/where the percentage change in mass is 0. Look up the water potential for that concentration of solution in, for example, a text book to give you the water potential of the potato cells.

4. Active Transport

Page 113 — Application Questions

- Q1 a) As the rate of sodium ion active transport increases, so does the rate of oxygen consumption.
b) Sodium ion active transport requires energy from ATP. As the rate of active transport increases, the rate of aerobic respiration must also increase in order to produce more ATP, which means the rate of oxygen consumption must increase too.
c) E.g. the rate of glucose consumption.
- Q2 a) The I⁻ ion because it needs to move from an area of lower concentration to an area of higher concentration.
b) E.g. the co-transporter binds to an I⁻ ion and a Na⁺ ion. The Na⁺ ion moves across the membrane into the thyroid gland down its concentration gradient. This moves I⁻ across the membrane into the cell too, against its concentration gradient.

Page 113 — Fact Recall Questions

- Q1 A hydrolysis reaction occurs which splits ATP into ADP and P_i/inorganic phosphate.
- Q2 a) A molecule attaches to a carrier protein in the membrane. The protein then changes shape and releases the molecule on the opposite side of the membrane. The process requires energy.
b) Co-transporters bind two molecules at a time. The concentration gradient of one of the molecules is used to move the other molecule against its own concentration gradient.
- Q3 Because sodium ions diffuse from the lumen of the ileum into the intestinal epithelium cells down their concentration gradient, through a sodium-glucose co-transporter protein. At the same time, the co-transporter carries glucose into the epithelium cell against its concentration gradient. Glucose is then able to diffuse into blood from the epithelial cell.
- Q4 increase

Exam-style Questions — pages 115-116

- 1.1 Figure 2 because the lower water potential of the salt solution has caused water to move out of the cells down the water potential gradient (**1 mark**). This has reduced the volume of the cells' cytoplasms (**1 mark**).
- 1.2 Cell A because its membrane has the largest surface area, which increases the rate of osmosis (**1 mark**).
- 1.3 The phospholipids are arranged in a bilayer (**1 mark**) with the hydrophilic heads facing outwards (**1 mark**) / the hydrophobic tails facing inwards (**1 mark**).
- 1.4 glycoproteins (**1 mark**)
- 1.5 Cholesterol is responsible for giving cells rigidity / helping to maintain the shape of cells (**1 mark**). Onion cells / plant cells have a cell wall which provides them with rigidity / helps to maintain their shape so they don't need as much cholesterol in their membranes as animal cells (**1 mark**).
- 2.1 The movement of molecules usually from a low to high concentration (**1 mark**) using energy (from ATP) to do so (**1 mark**).
- 2.2 It is a carrier protein/co-transporter (**1 mark**), which binds glucose and sodium ions at the same time (**1 mark**).
- 2.3 The centre of the phospholipid bilayer is hydrophobic (**1 mark**). It forms a barrier to the diffusion of water-soluble substances including most polar molecules (**1 mark**). Glucose is a polar molecule that can't diffuse directly across the membrane (**1 mark**).
- 3.1 E.g. in case the cubes did not all start out at exactly the same mass (**1 mark**). / To enable a fair comparison between the cubes (**1 mark**).
- 3.2 16% (accept 15-17%) (**1 mark**)
Don't forget that pure water is always 0 kPa.
- 3.3 The water potential in these three solutions must have been lower than the water potential of the potato cells (**1 mark**) so water moved out of the cells by osmosis (**1 mark**).
- 3.4 -425 kPa (accept any answer between -400 and -450 kPa) (**1 mark**)
Remember, all you need to do is read the water potential off the graph where the change in mass equals zero.
- 3.5 E.g. they could do repeats of the experiment for each concentration of sucrose solution and calculate a mean percentage change in mass (**1 mark**).
There's more on precise results in the Practical and Maths Skills section at the front of this book.
- 3.6 Before 12 hours (**1 mark**) because the rate of osmosis will be faster due to the increase in surface area (**1 mark**).

Topic 2C: Cells and The Immune System

1. Antigens

Page 117 — Fact Recall Questions

- Q1 The molecules found on the surface of cells that can generate an immune response when detected by the body.
Q2 The immune system identifies them as foreign.

2. The Immune Response

Page 121 — Application Questions

- Q1 a) Mouse A had 10 units, Mouse B had 10 000 units.
b) Mouse B was already immune. You can tell this because the immune response was much quicker and stronger than the immune response of Mouse A.
c) i) Day 20
ii) The mouse's memory B-cells rapidly divided into plasma cells that produced the antibody needed to bind to the antigen. The mouse's memory T-cells rapidly divided into the correct type of T-cells to kill the cell carrying the antigen.
- Q2 Antibodies will be generated against antigens on the surface of *S. pyogenes*. These will then bind to antigens on the surface of heart cells because the antibodies are very close to a complementary shape to the heart cell antigens. The immune system would then attack the heart cells and cause rheumatic fever.
The command word in this question is 'suggest', so you're not expected to know the exact answer. You're expected to use what you know about the immune system to come up with a possible explanation.

Page 121 — Fact Recall Questions

- Q1 When activated by antigens presented by phagocytes, helper T-cells release chemical signals to activate phagocytes, cytotoxic T-cells and B-cells.
Q2 The function of plasma cells is to produce antibodies.
Q3 The cellular immune response involves the T-cells and other immune cells they interact with e.g. phagocytes. The humoral response involves B-cells, clonal selection and the production of monoclonal antibodies.
Q4 E.g. the primary response happens the first time a pathogen invades, the secondary response happens the second time a pathogen invades. / The primary response involves B and T-cells, the secondary response also involves memory cells. / There are symptoms with a primary response, but not with a secondary response.

3. Immunity and Vaccines

Page 124 — Application Questions

- Q1 75% (accept answers in the range of 74-76%)
Q2 1000 cases
Q3 The number of cases decreased in a fluctuating pattern from a peak of around 6000 cases in 1960 to a peak of nearly 2000 cases around 1975. This is because more people were directly protected by the vaccine, and some people were protected by herd immunity.
Q4 a) Initially it increased slightly to about 80% of the population, and then decreased to around 50%.
b) The number of cases increased from a peak of around 2000 cases in 1975 to a peak of around 4500 cases in 1983. This is because fewer people were directly protected by vaccination and fewer people were indirectly protected by herd immunity.

The question asks you to explain, so you need to give reasons why the decreased uptake of the vaccine caused the change in the number of cases.

Page 124 — Fact Recall Questions

- Q1 Active immunity is the type of immunity you get when your immune system makes its own antibodies after being stimulated by an antigen.
Passive immunity is the type of immunity you get from being given antibodies made by a different organism — your immune system doesn't produce any antibodies of its own.
Q2 Vaccines contain antigens that cause your body to produce memory cells against a particular pathogen. This makes you immune.
Q3 Herd immunity is where unvaccinated people are protected because the occurrence of the disease is reduced by the number of people who are vaccinated.
Q4 Any two from: e.g. all vaccines are tested on animals and some people disagree with animal testing. / Testing vaccines on humans can be risky. / Some people don't want to take vaccines due to the risk of side effects, but they are still protected by herd immunity, which other people think is unfair. / If there was an epidemic of a new disease deciding who would receive a vaccine would be difficult.

4. Antigenic Variation

Page 125 — Fact Recall Questions

- Q1 Antigenic variation is when the antigens on the surface of a pathogen change.
Q2 If the influenza virus undergoes antigenic variation the memory cells produced from the first infection will not recognise the different antigens. The immune system has to carry out a primary response to the new antigens. This takes time to get rid of the infection, which is why you get ill again.

5. Antibodies in Medicine

Page 129 — Application Questions

- Q1 a) i) A
ii) O

Person 1 had a positive result with anti-antigen A because it reacted with the antigen A in her blood, but a negative result with anti-antigen B because she doesn't have any B antigens for it to react with. That means Person 1 must be blood type A. Person 3 had two negative results because they don't have A or B antigens — so they must be blood type O.

- b) i) No
ii) Yes

- Q2 a) To remove any unbound secondary antibody, so there won't be a false positive result if there are no primary antibodies present.
b) The substrate will change colour, because there will have been (primary) antibodies to the gluten protein in the patient's serum. These will have bound to the gluten protein in the well, and then the secondary antibody will have bound to them. This means the enzyme that the substrate reacts with will be present in the well.
c) E.g. to reduce the likelihood of getting a false result / to reduce the effect of random error / to make the results more precise.
d) The control using antibodies should show a positive result (it's a positive control) — it shows that the secondary antibody will bind to an antibody specific to the gluten protein / that the enzyme will react with the substrate, so the result will be a colour change. The control using salt solution should show a negative result (it's a negative control) — it shows that all unbound secondary antibody is removed by washing the well plate / that the colour change is due to the presence of the enzyme and nothing else, so the result will be no colour change.

6. Interpreting Data About Vaccines and Antibodies

Page 131 — Application Questions

Q1 $12 \times 61 = 732$

Q2 Minor reactions are about five times more common than serious reactions. Serious reactions are about 120 times more common than Guillain-Barré syndrome.

Rather than just saying it's more common, work out how much more common it is — manipulating data gets you higher marks in the exam.

Q3 No it does not support the idea that the influenza A vaccine increases the risk of Guillain-Barré syndrome.

If the background rate is 1 per 100 000 people you would expect to see 10 cases per million people. The study only showed a rate of 0.1 cases per million people, which is far below the background rate.

7. HIV and Viruses

Pages 134-135 — Application Questions

- Q1 a) 1995 because, e.g., after this year the number of people diagnosed with AIDS fell and the number of people living with HIV infection increased rapidly.
b) Number of AIDS deaths in 1995 (thousands) = 50
Number of AIDS deaths in 1998 (thousands) = 20
Percentage change = $\frac{\text{final value} - \text{original value}}{\text{original value}} \times 100$
 $= -0.6 \times 100 = -60\%$

So percentage decrease = **60%**

- c) People with HIV develop AIDS when the number of helper T-cells in their bodies falls to a critically low level. HAART reduces the amount of HIV in the body, so the virus takes longer to destroy the same number of helper T-cells. This may increase the length of time it takes for HIV to progress to AIDS.

- Q2 a) E.g. the virus infects and destroys helper T-cells that stimulate a B-cell response (to produce antibodies), so the overall immune response could be slower/ less efficient. / The antibodies produced by B-cells cannot reach the virus if it has entered a helper T-cell/becomes latent, so the immune response is less effective.
b) The attachment proteins are antigens on the HIV membrane. The anti-HIV antibodies produced are complementary to the antigen, so if it changes shape the antibodies won't recognise it / bind to it.

Page 135 — Fact Recall Questions

Q1 HIV stands for human immunodeficiency virus.

Q2 It causes AIDS (Acquired Immunodeficiency Syndrome).

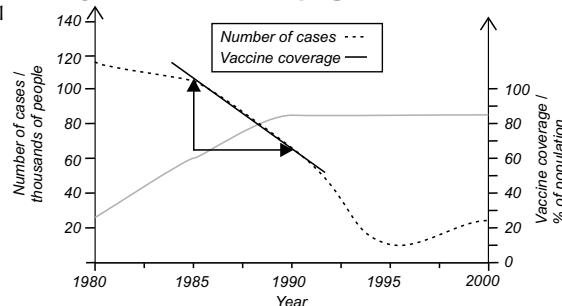
Q3 A = capsid, B = envelope, C = attachment/envelope protein, D = RNA

Q4 The viral attachment protein attaches to a receptor molecule on the cell membrane of the host helper T-cell. The capsid is then released into the cell, where it uncoats and releases RNA into the cell's cytoplasm. In the cell, reverse transcriptase is used to make a complementary strand of DNA from the viral RNA. Double-stranded DNA is made from this, which is inserted into the human DNA. Enzymes in the cell are used to make viral proteins from the inserted viral DNA. The viral proteins are assembled into new viruses, which bud from the cell and go on to infect other cells.

Q5 Antibiotics are designed to target bacterial enzymes and ribosomes, which are different to those in humans. Viruses do not have their own enzymes and ribosomes (they use those found in the host), so cannot be targeted in this way.

Exam-style Questions — pages 137-138

1.1



$$\begin{aligned} \text{Rate} &= \text{change in } y \div \text{change in } x \\ &= (105 - 65) \div (1990 - 1985) = 40 \div 5 \\ &= \mathbf{8 \text{ cases / thousands of people year}^{-1}} \\ &\quad (\text{Accept any answer between } 7 \text{ cases/thousands of people year}^{-1} \text{ and } 9 \text{ cases / thousands of people year}^{-1}.) \end{aligned}$$

(2 marks for the correct answer, 1 mark for evidence of the correct calculation)

- 1.2 The evidence does not support the conclusion. The data is for the whole world, not for the UK, so the pattern may not be true for the UK (**1 mark**). The data covers between 1980-2000, not up to 2011 so the pattern may not be true in 2011 (**1 mark**).
- 1.3 Any sensible answer, e.g. antigenic variation makes the vaccine ineffective (**1 mark**).
- 1.4 It prevents them from suffering from the disease because the antibodies bind to the toxin and prevent it from causing muscle spasms (**1 mark**). The injection does not contain pathogen antigens, so does not stimulate the production of memory cells (**1 mark**).
- 2.1 When a phagocyte recognises the antigens on a pathogen, the cytoplasm of the phagocyte moves around the pathogen, engulfing it (**1 mark**). The pathogen is now contained in a phagocytic vacuole inside the phagocyte (**1 mark**). A lysosome fuses with the phagocytic vacuole (**1 mark**) and the lysozymes inside the lysosome break down the pathogen (**1 mark**).
- 2.2 Any five from: e.g. The phagocytes present foreign antigens from engulfed pathogens on their surface (**1 mark**). Receptor proteins on the surface of helper T-cells bind to the antigens, activating the T-cells (**1 mark**). Activated helper T-cells release chemical signals that activate phagocytes, cytotoxic T-cells and B-cells (**1 mark**). When B-cells, which are covered in antibodies, meet an antigen with a complementary shape they bind to it (**1 mark**). This, along with chemical signals released from helper T-cells, activates the B-cells (**1 mark**). This is clonal selection (**1 mark**). The B-cells then divide into plasma cells (**1 mark**). The plasma cells then produce antibodies specific to the antigen (monoclonal antibodies) (**1 mark**).
- [Maximum of 5 marks available.]**
- 3.1 They're antibodies produced from a single group of genetically identical B-cells / plasma cells (**1 mark**). They're specific because their binding sites have a unique tertiary structure (**1 mark**) that only an antigen with a complementary shape can fit into (**1 mark**).
- 3.2 An antibody has variable regions where the antigen binds (**1 mark**). Each antibody has a different shaped variable region with a different tertiary structure (**1 mark**). They also have a constant region that is the same in all antibodies (**1 mark**). An antibody consists of light chains and heavy chains that are joined together by disulfide bridges (**1 mark**).

- 3.3 Bupropion has a similar structure to amphetamine (**1 mark**). So it may bind to the antibody that is complementary to amphetamine, causing a positive result (**1 mark**).
- 3.4 E.g. monoclonal antibodies are made using animal cells, and some people disagree with using animals this way (**1 mark**).
- 4.1 After the first infection their T-cells and B-cells produced memory cells (**1 mark**). When they were exposed for a second time the memory B-cells divided into plasma cells that produced the right type of antibodies to destroy the virus (**1 mark**). The memory T-cells divided into the correct type of T-cells to quickly destroy the virus (**1 mark**).
- 4.2 E.g. helper T-cells release chemical signalling molecules to activate cytotoxic T-cells/phagocytes/B-cells (**1 mark**). If the Spanish flu patients' helper T-cells released too many signalling molecules, it could have resulted in the over-activation of these other immune cells / more of these other immune cells to be activated than normal (**1 mark**).
- 4.3 E.g. children and the elderly have weaker immune systems than young adults (**1 mark**) so fewer cytotoxic T-cells/phagocytes/B-cells were activated (**1 mark**).
- 4.4 The neuraminidase and haemagglutinin antigens on the Asian flu strain were different from the antigens on the Spanish flu strain (**1 mark**), so any memory cells created against H1N1 would not detect H2N2 (**1 mark**). So the immune system would have to start from scratch and carry out a primary immune response if exposed to Asian flu (**1 mark**).

Topic 3

Topic 3A: Exchange and Transport Systems

1. Size and Surface Area

Page 140 — Application Question

- Q1 a) i) A — surface area = $6 \times 2 \times 2 = 24 \text{ cm}^2$
B — surface area = $(4 \times 4 \times 2) + (2 \times 2 \times 2)$
 $= 32 + 8 = 40 \text{ cm}^2$
C — surface area = $4\pi r^2$
 $= 4 \times \pi \times 2.5^2$
 $= 79 \text{ cm}^2$ (2 s.f.)
- ii) A — volume = $2 \times 2 \times 2 = 8 \text{ cm}^3$
B — volume = $2 \times 4 \times 2 = 16 \text{ cm}^3$
C — volume = $\frac{4}{3}\pi r^3$
 $= \frac{4}{3}\pi \times 2.5^3$
 $= 65 \text{ cm}^3$ (2 s.f.)
- iii) A — SA:V = 24:8 (or 3:1)
B — SA:V = 40:16 (or 5:2 or 2.5:1)
C — SA:V = 79:65 (or 1.2:1)

You should have got the same answers for shape C whether you used π as 3.14 or the π button on your calculator. If an exam question specifies which value to use for π , make sure you do what it says.

b) A

Simplify all of the ratios to 1 in order to compare them, e.g. A = 3:1, B = 2.5:1 and C = 1.2:1 — it's then obvious that A is the largest ratio.

Pages 142-143 — Application Questions

- Q1 a) Adélie penguin. The Adélie penguin would have the larger surface area : volume ratio because it's smaller than the Emperor penguin.
- b) The Emperor penguin because its large size means it has a lower surface area to volume ratio than the Adélie penguin. This means it's harder for it to lose heat from its body / easier for it to retain heat in its body, so it'll be better suited to living in colder regions than the Adélie penguin.
- c) The Adélie penguin because it has a more compact shape, and so has a lower surface area : volume ratio than the Rockhopper penguin. Therefore it won't lose heat as easily so it'll be better suited to living in colder regions than the Rockhopper penguin.
- Q2 Small animals have a high surface area : volume ratio meaning they will lose heat easily in cold temperatures. Underground temperatures will be warmer than on the surface, so they go underground to keep warm.
- Q3 Small birds. Smaller birds have a higher surface area : volume ratio so they will lose heat more quickly than larger birds. Therefore they are more likely to have adaptations to keep warm.
- Q4 Large animals have a low surface area : volume ratio so they find it hard to lose heat. They are active at night because it is cooler.

Page 143 — Fact Recall Questions

- Q1 a) Any two from: e.g. oxygen / nutrients / water.
b) E.g. carbon dioxide, urea.
- Q2 Lower surface area : volume ratio.
- Q3 Some cells are deep within the body so the distance between them and the outside environment is too great for diffusion to take place quickly. Larger animals have a low surface area : volume ratio. This means they don't have a large enough area exposed to the environment to be able to exchange all the substances they need quickly enough using diffusion.
- Q4 A system in a multicellular organism that carries substances to and from individual cells.
- Q5 High surface area : volume ratio.
- Q6 An animal with a compact shape has a low surface area : volume ratio. This means they lose less heat. An animal with a less compact shape has a higher surface area : volume ratio. This means they lose heat more easily.
- Q7 Any two from: e.g. they might have a higher metabolic rate. / They might hibernate. / They might have thick layers of fur.
- Q8 Any two from: e.g. they might spend a lot of time in water. / They might have features that increase their surface area, e.g. large ears.

2. Gas Exchange

Page 147 — Application Questions

- Q1 B. E.g. the leaf is curled with the stomata inside, protecting them from the wind so less water is lost. / There are lots of hairs on the epidermis to trap water vapour, reducing the concentration gradient of water between the leaf and the air. / The stomata are sunken in pits to trap water vapour, reducing evaporation by lowering the concentration gradient.
- Q2 A concentration gradient would still be maintained between the water and the blood, but it would be less steep. This means the fish wouldn't be able to take in as much oxygen as they would in clean water.
- Q3 a) It increases steadily.
To answer this question you need to look at the arrow head of the red line — it's pointing upwards so the oxygen concentration of the blood is increasing.
b) It decreases steadily.
c) 80%
d) Because at point X the oxygen concentration of the water is higher than in the blood (about 92 %) — so oxygen has diffused into the blood down its concentration gradient.

Page 147 — Fact Recall Questions

- Q1 Any two from: they have a large surface area. / They're thin. / A steep concentration gradient is maintained across them.
- Q2 Single-celled organisms can exchange gases directly through their cell-surface membrane. This has a large surface area, is thin and has a short diffusion pathway, so there's no need for a gas exchange system.
- Q3 Each gill is made of lots of thin plates called gill filaments. These are covered in lots of tiny structures called lamellae. Lamellae have a thin surface layer of cells and a good blood supply.
- Q4 The counter-current system works by maintaining a steep concentration gradient between the water and the blood along the entire length of the gill. Blood flows through the lamellae in one direction and water flows over the lamellae in the opposite direction. This means that water with a relatively high oxygen concentration always flows next to blood with a lower oxygen concentration. Oxygen then diffuses into the blood from the water down the concentration gradient.
- Q5 The surface of the mesophyll cells in the leaf.
- Q6 Through the stomata in the epidermis.
- Q7 Through the spiracles on the surface of the insect's body.
- Q8 Carbon dioxide from the cells moves down its concentration gradient through the tracheoles towards the spiracles to be released into the atmosphere.
- Q9 A plant specially adapted for life in a warm, dry or windy habitat.
- Q10 Any three from: stomata sunk in pits / curled leaves with stomata inside / a layer of hairs on the epidermis / a reduced number of stomata / waxy, waterproof cuticles on leaves and stems.

3. Gas Exchange in Humans

Page 151 — Application Questions

- Q1 a) 1
b) A
c) Speed = distance ÷ time = $0.82 \div 2 = 0.41 \text{ mm s}^{-1}$.
Always double-check the question to see if it tells you what units to use in your answer. If it doesn't say then make sure you pick a sensible unit.
- Q2 Less air, and so less oxygen, would be inhaled in each breath. This means the concentration gradient of oxygen between the alveoli and the capillaries will be less steep, slowing the rate of diffusion.

Page 151 — Fact Recall Questions

- Q1 The external intercostal and diaphragm muscles contract, which causes the ribcage to move upwards and outwards and the diaphragm to flatten.
- Q2 During forced expiration, the external intercostal muscles relax and internal intercostal muscles contract, pulling the ribcage further down and in.
- Q3 Oxygen diffuses out of the alveoli, across the alveolar epithelium and the capillary endothelium, and into haemoglobin in the blood.
- Q4 Alveoli have a thin exchange surface, which means there's a short diffusion pathway. This speeds up the rate of diffusion into the blood. There is a large number of alveoli so there is a large surface area for gas exchange, which speeds up the rate of diffusion. There's also a steep concentration gradient of oxygen and carbon dioxide between the alveoli and the capillaries, which increases the rate of diffusion. This is constantly maintained by the flow of blood and ventilation.

4. The Effects of Lung Disease

Page 155 — Application Questions

- Q1 Graph A, because the tidal volume is much lower.
- Q2 a) The alveoli are enlarged/much larger in the diseased lungs than in the healthy lungs. / The alveoli in the diseased lungs have merged together. In the healthy lungs they're more distinct.
- b) Having enlarged alveoli means there's a smaller surface area for gas exchange, slowing the rate of diffusion of oxygen into the blood. So a patient with emphysema would have a lower level of oxygen in the blood.
- Q3 a) The data shows that before inhaling salbutamol, the median area of a bronchial cross-section in healthy volunteers was bigger than in the asthmatics — 29 mm^2 compared to 10 mm^2 . Inhaling salbutamol reduced the area of the cross-section in healthy volunteers by 2 mm^2 , but in asthmatics the area of the cross-section almost doubled to 18 mm^2 .
- b)
- $$\begin{aligned}\text{percentage change} &= \frac{\text{final} - \text{original}}{\text{original}} \times 100 \\ &= \frac{18 - 10}{10} \times 100 \\ &= 80\%\end{aligned}$$
- c) Salbutamol could be used in inhalers to relax the smooth muscles lining the bronchioles in asthmatics. During an asthma attack, the smooth muscle contracts, causing constriction of the airways. The graph shows that after inhaling salbutamol the bronchioles aren't as constricted, so the salbutamol must relax the muscles.

Page 155 — Fact Recall Questions

- Q1 The maximum volume of air it is possible to breathe forcefully out of the lungs after a really deep breath in.
- Q2 Scar tissue is thicker and less elastic than normal lung tissue. This means that the lungs are less able to expand and so can't hold as much air as normal, so the tidal volume is reduced.
- Q3 Scar tissue is thicker than normal lung tissue, so diffusion of gases is slower.
- Q4 Reduced air flow means that FEV₁ is severely reduced (i.e. less air can be breathed out in 1 second).

5. Interpreting Lung Disease Data

Page 159 — Application Questions

- Q1 Male deaths due to COPD increased from just over 10 per 100 000 people in 1946 to almost 80 per 100 000 in 1972. It then slowly decreased to about 40 per 100 000 by 1998.
- Q2 E.g. between about 1948 and 1969 there doesn't seem to be any correlation between female deaths from COPD and tobacco consumption. After this year the number of female deaths from COPD increases as tobacco consumption decreases (there's a negative correlation). This isn't enough to say that COPD in women is not caused by smoking though. Tobacco consumption in women might have risen while tobacco consumption in the overall population was decreasing, but you can't tell from this data. Also, female deaths from COPD could be increasing for other reasons, e.g. industrial causes, even if tobacco consumption is still a cause of the disease.
- Q3 E.g. they could use it to help them decide whether there's a link between tobacco consumption and COPD and whether to impose legal restrictions on the sale/advertisement of tobacco products as a result.

6. Dissecting Gas Exchange Systems

Page 162-163 — Application Question

- Q1 a) Because she could end up sucking up stale air from inside the lungs into her mouth.
b) Put the lungs inside a plastic bag while she inflates them. This helps prevent bacteria being released into the air.
c) The lungs would deflate by themselves because of the elastin in the walls of the alveoli.
d) Lengthways, down the gaps in the C-shaped rings of cartilage.

Page 163 — Fact Recall Questions

- Q1 E.g. check that they are clean, sharp and free from rust.
Q2 E.g. some people believe that it is morally wrong to kill animals just for dissections. Animals used for dissections may not be raised in a humane way/killed in a humane way.

Exam-style Questions — pages 164-165

- 1.1 A = lamellae (**1 mark**). Lamellae increase the surface area of the gill for diffusion (**1 mark**). They're also thin, which reduces the diffusion distance between the water and the blood (**1 mark**).
1.2 It has a counter-current exchange system (to maintain a large concentration gradient of oxygen between the water and the blood (**1 mark**).
1.3 Air enters through an insect's spiracles (**1 mark**). Oxygen then diffuses down the tracheae and tracheoles, directly into respiring cells (**1 mark**).
1.4 Any two from: they can close their spiracles to prevent water loss (**1 mark**). / They have a waxy, waterproof cuticle all over their body to reduce evaporation (**1 mark**). / They have hairs around their spiracles to trap moist air and reduce evaporation (**1 mark**).
2.1 width of alveolus = width of image ÷ magnification
= 9 mm ÷ 60
= 0.15 mm × 1000 (to convert to micrometres)
= 150 µm
(1 mark for correct calculation,
2 marks for correct answer)
The question tells you to give your answer in µm, so you need to remember to convert your answer from mm to µm. If you're a bit rusty on this, check out p. 80.
2.2 E.g. the walls of the alveoli have been destroyed in the diseased alveoli (**1 mark**). Destruction of the alveolar walls reduces the surface area of the alveoli (**1 mark**), so the rate of gaseous exchange would decrease (**1 mark**).
2.3 There would be a steeper concentration gradient of oxygen between the alveoli and the capillaries (**1 mark**). This would increase the rate of diffusion of oxygen into the blood (**1 mark**).
3.1 E.g. peak expiratory flow rate is lower for the person with asthma/line B because the inflamed bronchioles restrict the amount of air that can pass through (**1 mark**). / The expiratory flow rate decreases more rapidly for the person with asthma/line B as more air is exhaled because the constricted bronchioles reduce the flow of air out of the lungs (**1 mark**). **(Accept reverse arguments about why the peak expiratory flow rate is higher for line A for 1 mark.)**
3.2 E.g. exercise may increase the strength of the respiratory muscles/the intercostal muscles and diaphragm (**1 mark**). This would allow air to be exhaled more forcefully, increasing the peak expiratory flow (**1 mark**).

- 4 In inspiration, the external intercostal and diaphragm muscles contract (**1 mark**). This causes the ribcage to move upwards and outwards and the diaphragm to flatten, increasing the volume of thoracic cavity (**1 mark**). As the volume of the thoracic cavity increases, the lung pressure decreases (to below atmospheric pressure), causing air to flow into the lungs (**1 mark**). In expiration, the external intercostal and diaphragm muscles relax (**1 mark**). The ribcage moves downwards and inwards and the diaphragm curves upwards/becomes dome-shaped again (**1 mark**). The volume of the thoracic cavity decreases, causing the air pressure to increase (to above atmospheric pressure), forcing air out of the lungs (**1 mark**).

5.1 surface area = $4\pi r^2$
= $4 \times \pi \times 0.7^2$
= $6 \mu\text{m}^2$ (1 s.f.)
volume = $\frac{4}{3} \pi r^3$
= $\frac{4}{3} \pi \times 0.7^3$
= $1 \mu\text{m}^3$ (1 s.f.)

surface area : volume = **6 : 1**

(2 marks for the correct ratio. 1 mark for either 6 or 1.)

- 5.2 Because it is a single-celled organism with a short diffusion pathway (**1 mark**) and a large surface area : volume ratio (**1 mark**). This means it can exchange substances quickly across its cell-surface membrane/outer surface (**1 mark**). To help you answer this question, think about why multicellular organisms do have a gas exchange system — it's because the diffusion pathway is too big and they have a small surface area : volume ratio, which makes diffusion too slow.

Topic 3B — More Exchange and Transport Systems

1. Digestion and Absorption

Page 169 — Application Questions

- Q1 On the cell membranes of epithelial cells lining the ileum.
Q2 water, glucose, fructose
Q3 Sucrase catalyses the hydrolysis of sucrose into glucose and fructose. These smaller molecules/monosaccharides can then be absorbed across the ileum epithelium into the bloodstream.
Make sure you write the names of enzymes and their substrates really clearly — if the examiner mistakes an 'o' for an 'a' (so thinks you've written sucrase rather than sucrose) you may lose marks.

Page 169 — Fact Recall Questions

- Q1 A reaction that breaks bonds though the addition of water.
Q2 lipases
Q3 Once lipids have been broken down by lipase, the monoglycerides and fatty acids stick with the bile salts to form micelles.
Q4 Exopeptidases act to hydrolyse peptide bonds at the ends of protein molecules. They remove single amino acids from proteins.
Q5 The micelles break up, releasing the monoglycerides and fatty acids, which diffuse directly across the membrane because they are lipid soluble.
Q6 Sodium ions are actively transported out of the epithelial cells into the ileum itself. They then diffuse back into the cells through sodium-dependent transporter proteins in the epithelial cell membranes, carrying amino acids with them.

2. Haemoglobin

Page 174 — Application Questions

- Q1 a) B. The dissociation curve would be further to the right after a bike ride than whilst watching television, because during the bike ride the man's respiration rate would have increased, raising the pCO_2 . This increases the rate of oxygen unloading so the dissociation curve shifts right.
- b) The Bohr effect.
- Q2 Badger — A. In an underground sett the oxygen concentration will be low, so the badger's haemoglobin will have the highest affinity for oxygen compared to the other animals, so the dissociation curve is furthest to the left. The badger needs to be able to get any available oxygen at a low pO_2 . Its dissociation curve is furthest to the left meaning it loads oxygen more readily at a lower oxygen concentration.
- Bush dog — C. Above ground there will be more oxygen than underground, so the bush dog's haemoglobin will have a lower affinity for oxygen than the badger's haemoglobin. The bush dog is more active than the brown-throated sloth, so it has a greater oxygen demand. This means its haemoglobin will also have a lower affinity for oxygen than the brown-throated sloth's, so its dissociation curve is furthest to the right.
- Brown-throated sloth — B. Above ground there will be more oxygen than underground, so the brown-throated sloth's haemoglobin will have a lower affinity for oxygen than the badger's haemoglobin. However, the brown-throated sloth's oxygen demand won't be as high as the bush dog's, as the brown-throated sloth is less active. This means the brown-throated sloth's haemoglobin will have an affinity for oxygen (and therefore dissociation curve) that is between the badger's and the bush dog's.

Page 174 — Fact Recall Questions

- Q1 To carry oxygen around the body.
- Q2 In red blood cells.
- Q3 four
- Q4 Loading describes oxygen binding with/joining to haemoglobin, and unloading describes oxygen being released from/leaving haemoglobin.
- Q5 oxyhaemoglobin
- Q6 How saturated haemoglobin is with oxygen at any given partial pressure of oxygen.
- Q7 It changes the shape of haemoglobin in a way that makes it easier for other oxygen molecules to join too.
- Q8 In the alveoli / lungs. This is the site where oxygen first enters the blood so it has the highest concentration of oxygen.

3. The Circulatory System

Page 176 — Application Question

- Q1 A — Vena cava
B — Renal vein
C — An arteriole
D — Renal artery
E — Aorta

Relative blood pressure is highest in the aorta as it has just left the heart. Relative blood pressure in the other blood vessels decreases as they get further away from the heart. The vessel with the lowest relative blood pressure is the vena cava as it is the last blood vessel before blood returns to the heart.

Page 178 — Application Questions

- Q1 a) i) From the blood into the tissue fluid.
ii) E.g. water, oxygen and nutrients (like glucose and amino acids)
- b) i) Venule end of the capillary bed.
ii) Arteriole end of the capillary bed.
- Q2 The water potential of the capillary is higher because there is less albumin in the blood. This means less water is absorbed by osmosis back into the capillary at the venule end of the capillary bed, which leads to an increase in tissue fluid.

Page 178 — Fact Recall Questions

- Q1 Mammals have a low surface area : volume ratio so they need a specialised mass transport system/a circulatory system to carry raw materials from specialised exchange organs to their body cells.
- Q2 pulmonary vein, vena cava
Remember, that the blood vessels that carry blood into the heart do not supply the heart muscle with blood — the blood goes into the chambers of the heart. Only the coronary vessels supply the heart muscle itself.
- Q3 pulmonary artery
- Q4 renal artery
- Q5 The (left and right) coronary arteries.
- Q6 A — vein, B — capillary, C — artery, D — arteriole
- Q7 An artery has a thick muscular wall with elastic tissue and a folded endothelium.
- Q8 A blood vessel that branches off from an artery.
- Q9 a) veins
b) To stop the backflow of blood.
- Q10 Any two from: the walls are only one cell thick / they're always very near the cells in exchange tissues / there's a large number of them in exchange tissues.
- Q11 The fluid that surrounds cells in tissues.
- Q12 a) At the arteriole end the hydrostatic pressure inside the capillaries is higher than the hydrostatic pressure in the tissue fluid. This means fluid is forced out of the capillaries and into the spaces around the cells, forming tissue fluid.
- b) At the venule end of the capillary bed, the water potential is lower in the capillary than it is in the tissue fluid. This means that some water re-enters the capillaries from the tissue fluid by osmosis.

4. The Heart

Page 183 — Application Questions

- Q1 The left atrium is contracting.
- Q2 Open. The left ventricle is contracting, so the pressure is higher in the ventricle than in the aorta, forcing the semi-lunar valve open.
- Q3 The left ventricle is relaxing.
- Q4 The left atrium is filling up.
- At point D, the increase in atrial pressure can't be due to the left atrium contracting because the diagram shows that the left ventricle is relaxing — i.e. the left ventricle doesn't contract next. So you need to think about what happens in the left atrium as the left ventricle is relaxing — it's filling up with blood to prepare for the next atrial contraction.
- Q5 Open. The ventricle is relaxing, increasing the volume and reducing the pressure in the chamber. The atrium has been filling, increasing the pressure in the chamber. So as the pressure in the atrium becomes higher than that in the ventricle, the atrioventricular valve will open.

Page 184 — Application Questions

- Q1 stroke volume \times heart rate = cardiac output
 $61 \times 79 = 4819 \text{ cm}^3 \text{ min}^{-1}$
- Q2 cardiac output \div stroke volume = heart rate
 $5075 \div 72.5 = 70 \text{ bpm}$
- Q3 cardiac output \div heart rate = stroke volume
 $5175 \div 75 = 69 \text{ cm}^3$

Page 184 — Fact Recall Questions

- Q1 right side
- Q2 A — pulmonary artery
B — aorta
C — inferior vena cava
D — pulmonary vein
E — right atrium
F — semi-lunar valve
G — right atrioventricular valve
H — left ventricle

- Q3 So that it can contract more powerfully, which means the blood can be pumped further and so travel all round the body. The less muscular right ventricle cannot contract as powerfully so cannot pump blood as far/only pumps blood to the lungs.
- Q4 a) semi-lunar valves
b) They stop blood flowing back into the heart after the ventricles contract.
- Q5 An ongoing sequence of contraction and relaxation of the atria and ventricles that keeps blood continuously circulating round the body.
- Q6 The volume of the atria decreases and the pressure increases.

5. Cardiovascular Disease

Pages 189-190 — Application Question

- Q1 a) It could decrease the number of new cases of CHD by 37 000 per year (accept 36 000 to 38 000).
Look carefully at the values on the y-axis — the projected annual change in the number of new cases of CHD is negative, which means there are fewer new cases of CHD.
- b) There'd be 59 000 fewer new cases of CHD by reducing BMI, but only 41 000 fewer new cases by reducing tobacco use/exposure — so between the two there'd be 18 000 fewer new cases by reducing BMI.
- c) Intervention 3 / reducing salt intake by 3 g per day. This is because this intervention is predicted to reduce the number of new cases of CHD per year by 110 000 — which is greater than any other intervention shown on the graph.
- d) i) The more salt intake is reduced by, the fewer new cases of CHD there are per year.
ii) A diet low in salt will decrease the risk of high blood pressure, which in turn will decrease the risk of damage to the coronary artery walls.
This means it's less likely that atheromas form in the coronary arteries, so the risk of CHD is reduced and it's likely that there'll be fewer new cases of CHD.

This question is asking you to explain why a low salt diet could lead to a lower risk of CHD — that's the opposite way round to how you've learnt it.

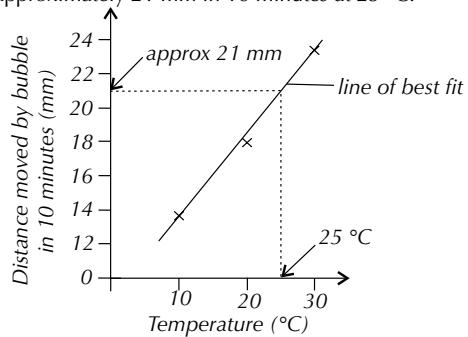
Page 190 — Fact Recall Questions

- Q1 An atheroma is a fibrous plaque formed from the build up and hardening of white blood cells, lipids and connective tissue.
- Q2 An atheroma partially blocks the lumen of an artery and restricts blood flow.
- Q3 a) High blood pressure increases the risk of damage to the artery walls. Damaged walls have an increased risk of atheroma formation.
b) E.g. any three from: atheroma formation / being overweight / not exercising / excessive alcohol consumption / high salt diet.
- Q4 a) E.g. any two from: diet high in saturated fat/salt / smoking / not exercising.
b) E.g. genetic predisposition / having high blood pressure as a result of another condition / some forms of diabetes / ethnicity / age / gender.

6. Transport in Plants — Xylem

Page 193 — Application Questions

- Q1 a) $10^\circ\text{C} - (15 + 12 + 14) \div 3 = 13.7 \text{ mm}$
 $20^\circ\text{C} - (19 + 16 + 19) \div 3 = 18 \text{ mm}$
 $30^\circ\text{C} - (25 + 22 + 23) \div 3 = 23.3 \text{ mm}$
- b) See graph below. The bubble would move approximately 21 mm in 10 minutes at 25°C .



- Q2 As the temperature increased, the distance moved by the bubble in 10 minutes increased too. This means the rate of transpiration increased with increasing temperatures, i.e. there is a positive correlation. At higher temperatures water molecules have more energy so they evaporate from the cells inside the leaf faster. This increases the water potential between the inside and outside of the leaf, making water diffuse out of the leaf faster.

Page 194 — Fact Recall Questions

- Q1 xylem vessels
- Q2 Water can move up a plant by cohesion and tension. Water evaporates from leaves at the top of the xylem. This creates tension which pulls more water into the leaf. As water molecules are cohesive, the whole column of water in the xylem moves upwards. More water then enters the stem through the roots.
- Q3 a) An increase in wind increases transpiration rate. Lots of air movement blows away water molecules from around the stomata. This increases the water potential gradient, which increases the rate of transpiration.
b) Light intensity, temperature and humidity.
- Q4 E.g. use a scalpel/razor blade to cut a thin cross-section of the stem. Place it in water to stop it drying out. Add a drop of water to a microscope slide and carefully add the stem section. Add a drop or two of stain (such as TBO) and leave for a short amount of time. Carefully apply a cover slip.

7. Transport in Plants — Phloem

Page 199 — Application Questions

- Q1 a) E.g. meristems / leaves.
b) i) At the roots active transport is used to actively load solutes/assimilates into the sieve tubes. This lowers the water potential inside the sieve tubes, so water enters the tubes by osmosis.
Remember, water always flows from a higher water potential to a lower water potential.
ii) At the sink solutes/assimilates are removed from the phloem to be used up. This increases the water potential inside the sieve tubes, so water leaves the tubes by osmosis.
- Q2 E.g. the radioactive carbon has been incorporated into organic substances produced by the plant during photosynthesis. The results show that these substances have been moved from the source in the leaves towards the sink in the roots because only the affected leaf and the upper part of the stem are black on the autoradiograph. This supports the mass flow hypothesis because solutes have been transported from areas of high pressure in the leaves to areas of lower pressure towards the root end of the stem. The solutes have not travelled into the other leaves, where the solute is also at a high concentration, because there is no pressure gradient in this direction.

Page 199 — Fact Recall Questions

- Q1 Organic solutes, e.g. sugars/sucrose, amino acids.
Q2 It's the movement of solutes/assimilates to where they're needed in a plant.
Q3 In a plant a source is where solutes/assimilates are made, whereas a sink is where solutes/assimilates are used up.
Q4 If a ring of bark is removed from a woody stem, a bulge forms above the ring. If the fluid from the bulge is analysed, it will have a higher concentration of sugars than the fluid from below the ring. This is because the sugars can't move past the area where the bark has been removed — this is evidence that there can be a downward flow of sugars.
Q5 Sugar travels to many different sinks, not just to the one with the highest water potential, as the model would suggest. / The sieve plates would create a barrier to mass flow. A lot of pressure would be needed for the dissolved substances to get through at a reasonable rate.

Exam-style Questions — pages 201-202

- 1.1 In the first stage the atria contract, which decreases their volume (**1 mark**) and therefore increases the pressure in the atria (**1 mark**).
1.2 Atrioventricular valves / AV valves (**1 mark**). They prevent the back-flow of blood into the atria when the ventricles contract (**1 mark**).
1.3 During stage one, the atrioventricular valves are open because the pressure in the atria is greater than that in the ventricles (**1 mark**). During stage two, the atrioventricular valves are closed because the pressure in the ventricles is greater than that in the atria (**1 mark**).
1.4 Because the atria are filling up with blood (**1 mark**).
1.5 pulmonary artery (**1 mark**)
1.6 The walls of the pulmonary artery have elastic tissue to stretch and recoil as the heart beats (**1 mark**). The inner lining/endothelium is folded, allowing the artery to stretch (**1 mark**). Both of these features help to maintain the high pressure of the blood as it leaves the heart (**1 mark**).

- 2.1 Reading off graph, distance moved by bubble in 5 minutes at 1.5 arbitrary units of light intensity = 15 mm
 $15 \div 5 = 3 \text{ mm min}^{-1}$
(2 marks for the correct answer, otherwise 1 mark for showing a calculation of 'distance ÷ time')
- 2.2 The lighter it gets, the wider stomata open (**1 mark**). This increases the evaporation rate from the leaves, which creates more tension in the xylem, pulling water into the leaves (**1 mark**). The whole column of water moves up the xylem because water molecules are cohesive (**1 mark**). The increased tension causes the water to move faster, meaning that the bubble moves further in a shorter amount of time (**1 mark**).
2.3 E.g. the experiment should be repeated with a light intensity of zero (**1 mark**).
3.1 Cardiovascular disease is a general term used to describe diseases associated with the heart and blood vessels (**1 mark**).
3.2 control group (**1 mark**)
3.3 E.g. by taking a larger sample size (**1 mark**). By making sure the men in the test group followed the dietary information (**1 mark**).
3.4 The fats are emulsified by bile salts, forming small droplets (**1 mark**). They are then broken down by lipases into monoglycerides and fatty acids (**1 mark**). The monoglycerides and fatty acids stick with the bile salts forming micelles (**1 mark**), which help move the monoglycerides and fatty acids towards the endothelium (**1 mark**). There they are released and diffuse directly across the membrane (**1 mark**) because they are lipid soluble (**1 mark**). **[Maximum of 3 marks available.]**
4.1 It is a protein with a quaternary structure consisting of four polypeptide chains (**1 mark**). Each polypeptide chain has a haem group containing an iron ion (**1 mark**).
4.2 Each animal's haemoglobin was 50% saturated at a lower pO_2 at high altitude than at sea level (**1 mark**). This means that haemoglobin has a higher affinity for oxygen/ unloads oxygen less readily at high altitudes than at sea level (**1 mark**). This suggests that the animals living at high altitudes live in environments with a lower oxygen concentration than those living at sea level (**1 mark**).
5.1 phloem tissue (**1 mark**)
5.2 The phloem is involved in translocation/the movement of organic solutes in a plant (**1 mark**). Translocation moves solutes from the source, where they are produced, to the sink, where they are used up (**1 mark**).
5.3 Cutting a C-shaped ring in the bark reduces the volume of the phloem, limiting the amount of solutes that can be transported (**1 mark**). The solutes normally travel from the leaves where they are produced, and flow through the plant (**1 mark**). Cutting a C-shaped ring in the bark means that more solutes are retained in the upper part of the plant where the fruits are produced, which may lead to greater fruit production (**1 mark**).

Topic 4

Topic 4A — DNA, RNA and Protein Synthesis

1. DNA

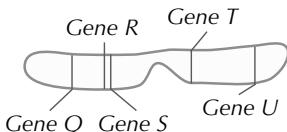
Page 203 — Fact Recall Questions

- Q1 Eukaryotic chromosomes are thread-like structures made up of a long molecule of DNA wound around proteins called histones. The DNA and proteins are then coiled up to form a chromosome.
- Q2 DNA in prokaryotic chromosomes is shorter than DNA in eukaryotic chromosomes and is also circular rather than linear. Also, the DNA isn't wound around histone proteins.

2. Genes and Chromosomes

Page 205 — Application Questions

Q1



Homologous chromosomes are the same so all of the genes should be in the same place.

Q2 a) ACTGTATTGATCGAACATGTCTA

This is the sequence of the exons only as these are the sections of the gene that actually determine the amino acid order.

b) 10

c) GC

Page 205 — Fact Recall Questions

- Q1 A sequence of DNA bases that codes for either a polypeptide or functional RNA.
- Q2 three
- Q3 The complete set of genes in the cell.
- Q4 The full range of proteins that the cell can produce.
- Q5 Introns and multiple repeats.
- Q6 exons
- Q7 A different form of a gene.
- Q8 A locus.

3. RNA and Protein Synthesis

Page 206 — Fact Recall Questions

- Q1 mRNA carries the genetic code from the DNA to the ribosomes.
- Q2 A group of three adjacent bases on an mRNA molecule.
- Q3 Messenger RNA
- Q4 Transfer RNA

4. Transcription and Translation

Page 208 — Application Questions

- Q1 It will inhibit protein synthesis. By inhibiting RNA polymerase, α -amanitin will prevent the transcription of mRNA from DNA, preventing protein synthesis from taking place.
- Q2 a) UCAAGCCUGCUCCGGCUACGAGCAUUU
b) 6
- One amino acid is coded for by three bases in an exon.

Page 210 — Application Questions

- Q1 E.g. it may affect the function of the ribosomes, preventing them from translating mRNA into amino acids. This could prevent/impair protein synthesis. You don't need to have learnt about Diamond-Blackfan anaemia to answer this question — so long as you know the process of translation, you can work out the answer.
- Q2 It could result in a shorter amino acid sequence being produced, which would change the primary structure of the protein and therefore the 3D tertiary structure of the protein. This could affect the protein's function. This could happen because translation of the mRNA sequence only continues until a stop signal is reached. Any codons after the stop signal would not be translated into amino acids.

Page 210 — Fact Recall Questions

- Q1 Transcription — takes place in the nucleus.
Translation — takes place at the ribosomes in the cytoplasm.
- Q2 a) an enzyme
b) transcription
- Q3 Because of complementary/specific base pairing.
- Q4 Eukaryotic DNA contains introns/regions that don't code for amino acids. These get transcribed into pre-mRNA along with the exons/coding regions. Splicing removes the introns from pre-mRNA and joins together the exons to create mRNA ready for translation into a protein.
- Q5 It doesn't contain introns.
- Q6 tRNA molecules carry amino acids to the ribosome during translation.
- Q7 ATP provides the energy needed for the bond between an amino acid and a tRNA molecule to form, allowing the tRNA to carry the amino acid to the ribosome.
- Q8 A tRNA molecule with an anticodon that's complementary to a codon on the mRNA attaches itself to the mRNA by complementary base pairing. A second tRNA molecule attaches itself to the next codon on the mRNA in the same way, and so on.
- Q9 Peptide bond

5. The Genetic Code and Nucleic Acids

Page 213 — Application Questions

- Q1 UACUUUCAAUAUGCGCAU
- Q2 TACAAAGTTGTCGCATGTAT
- Remember, DNA is a complementary sequence to mRNA and in DNA, T replaces U as a base.
- Q3 UACGUUAUGUAAAAGUU
- tRNA codons also have a complementary sequence to mRNA codons, but tRNA still has U as a base.
- Q4 Phe - Gln - Ile - His - Ala - Tyr
- To answer this question, work out what the complementary mRNA codons would be first then match up the appropriate amino acids using the table.
- Q5 The DNA sequence is missing the base triplets: CGC, TAT and GTT. The amino acid sequence is missing: Tyr and His.

Page 214 — Application Questions

- Q1 Uracil is present as a base in mRNA but not DNA, so uracil can be used as a marker for RNA synthesis.
- Q2 As the concentration of puromycin increased, the % inhibition of the development of respiration, leucine uptake and uracil uptake also increased. The development of respiration and leucine uptake were strongly inhibited, with uracil uptake being inhibited to a slightly lesser degree.
- Q3 protein synthesis

Page 214 — Fact Recall Questions

- Q1 Each triplet is read in sequence, separate from the triplet before it and after it — base triplets don't share their bases.
- Q2 A base triplet that tells the cell when to start production of a particular protein.
- Q3 universal

Exam-style Questions — page 216

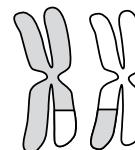
- 1.1 RNA polymerase lines up free RNA nucleotides alongside exposed bases on the DNA template strand (**1 mark**). The enzyme then moves along the DNA strand, assembling a complementary mRNA sequence from the RNA nucleotides by joining them together (**1 mark**).
- 1.2 Genes in eukaryotic DNA contain introns (sections that don't code for amino acids) (**1 mark**). After transcription the introns are removed from pre-mRNA strands by splicing, leaving only the exons (parts of the gene that code for amino acids) which form mRNA (**1 mark**). So eukaryotic mRNA would be shorter than the DNA it was transcribed from (**1 mark**).
- 2.1 The sequence of base triplets/codons in mRNA which code for specific amino acids (**1 mark**).
- 2.2 It is universal because the same specific base triplets code for the same amino acids in all living things (**1 mark**). It is degenerate because there are more possible combinations of triplets than there are amino acids (**1 mark**).
- 2.3 (DNA sequence: CCT GTG CGT GGA GTG)
tRNA anticodons: CCU GUG CGU GGA GUG
(2 marks for 5 correct tRNA anticodons. Allow 1 mark if tRNA anticodons are correct but T hasn't been replaced with U.)
Remember, tRNA is complementary to a strand of mRNA — so it's just like DNA but with U replacing T.
- 2.4 tRNA is folded into a clover shape and held together by hydrogen bonds whereas mRNA is not (**1 mark**). Three adjacent bases in mRNA form a codon whereas tRNA has three specific bases called an anticodon (**1 mark**). tRNA has an amino acid binding site whereas mRNA does not (**1 mark**).
- 2.5 ribosome (**1 mark**)
- 2.6 tRNA molecules carry amino acids to the ribosome (**1 mark**). A tRNA molecule with an anticodon that's complementary to the first codon on the mRNA attaches itself to the mRNA by complementary base pairing (**1 mark**). A second tRNA molecule attaches itself to the next codon on the mRNA in the same way and the two amino acids are joined by a peptide bond (**1 mark**). The first tRNA molecule moves away, leaving its amino acid behind and this process continues and produces a polypeptide chain (**1 mark**). Always look at the number of available marks — the more marks there are, the more detailed your answer should be.

Topic 4B — Diversity and Selection

1. Meiosis and Genetic Variation

Pages 221-222 — Application Questions

Q1



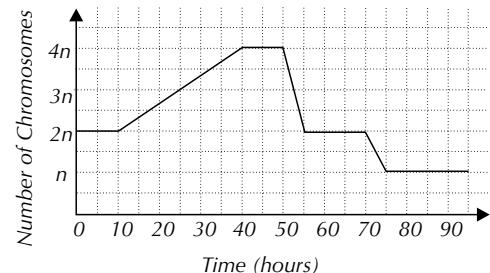
Q2 E.g.



You should have drawn a single chromatid from each homologous pair — so there shouldn't be two black chromatids and a grey chromatid, for example.

- Q3 a) After meiosis II, because there is only one chromatid of each chromosome.
b) Between meiosis I and meiosis II, because there are no homologous pairs, but each chromosome has two sister chromatids.
c) Before meiosis I, because there are homologous pairs of chromosomes.
- Q4 a) i) The DNA is being replicated to produce two copies of each chromosome.
ii) The DNA is condensing to form double-armed chromosomes and the chromosomes are arranging themselves into homologous pairs.
iii) Meiosis I occurs — the homologous pairs are separated halving the chromosome number.

b) E.g.



During this time period, meiosis II occurs and the sister chromatids are separated — halving the chromosome number again and generating haploid cells.

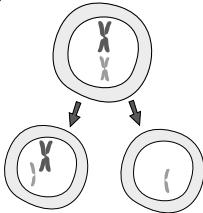
Page 222—Fact Recall Questions

- Q1 a) diploid
b) haploid
c) diploid
- Q2 a) The homologous pairs separate.
b) The sister chromatids separate.
- Q3 a) Crossing over and independent segregation of chromosomes.
b) Crossing over is when chromatids twist around each other and bits of chromatid swap over. The resulting chromosomes contain the same genes but now have a different combination of alleles. This means that when the chromatids separate at meiosis II, each of the four daughter cells will contain chromatids with different alleles.
Independent segregation is when the random separation of homologous pairs in meiosis I means that different combinations of maternal and paternal chromosomes go into each cell. This produces genetic variation in the gametes.
- Q4 Mitosis produces cells with the same number of chromosomes as the parent cell, whereas meiosis produces cells with half the number of chromosomes as the parent cell. In mitosis daughter cells are genetically identical to each other and to the parent cell, whereas in meiosis daughter cells are genetically different from one another and the parent cell. Mitosis produces two daughter cells whereas, meiosis produces four daughter cells.

2. Mutations

Page 225 — Application Questions

- Q1 a) G (in second triplet) has been deleted.
b) leucine – glutamic acid – tyrosine
leucine – serine
c) The genetic code is degenerate, which means that some amino acids are coded for by more than one DNA triplet. In this example, TAT undergoes a substitution and becomes TAC. Both TAT and TAC code for tyrosine so the amino acid sequence produced stays the same.
- Q2 a) chromosome non-disjunction
b) Non-disjunction would mean that chromosome 18 fails to separate properly during meiosis, so one cell gets an extra copy of 18 and another gets none. When the gamete with the extra copy fuses to another gamete at fertilisation, the resulting zygote will have three copies of chromosome 18.
c) E.g.



When the homologous pairs line up, the pair of sister chromatids making up chromosome 18 fail to separate.

Page 225 — Fact Recall Questions

- Q1 Changes in the DNA base sequence of chromosomes.
Q2 E.g. deletions
Q3 a) They increase the rate of mutations occurring. / They increase the probability of mutations occurring.
b) E.g. ultraviolet radiation / ionising radiation / X-rays / gamma rays

3. Genetic Diversity

Page 227 — Application Question

- Q1 a) Population 1 is more genetically diverse because it has a relatively high frequency of three different alleles for flower colour / because population 2 has fewer different alleles for flower colour.
b) Population 2 could have experienced an event in the past that caused a big reduction in its numbers. This could have meant that all the organisms with the purple allele (and lots with the pink allele) died before reproducing — causing the purple allele to be lost from the gene pool (and the frequency of the pink allele to be reduced). When the survivors reproduced, the new population would have been created without the purple allele (and a lower frequency of the pink allele).

Page 227 — Fact Recall Questions

- Q1 Genetic diversity is the number of different alleles of genes in a species or population.
Q2 By mutations in the DNA forming new alleles. By different alleles being introduced into a population when individuals from another population migrate into it and reproduce / gene flow.

4. Natural Selection

Pages 229-230 — Application Questions

- Q1 As the result of a mutation, some individuals in the population have a darker colouring that helps them to blend into their environment (wooded areas) better. This is beneficial because it helps them to avoid predators and sneak up on prey. So these individuals are more likely to survive, reproduce and pass on the allele for darker colouring. The frequency of the allele for darker colouring will increase in the population so that, after many generations, most organisms in the population will carry the allele for darker colouring.
- Q2 Some individuals in the population have a mutated allele that gives them resistance to DDT. The population is exposed to DDT, killing the mosquitoes without the resistance allele. Individuals with the resistance allele survive, reproduce and pass on the allele. The frequency of the resistance allele will increase in the population so that, after many generations, most organisms in the population will carry the allele for DDT resistance.
See, I told you. It's the same answer whatever the adaptation — it helps them to survive, reproduce and pass on the allele for that adaptation. The frequency of the beneficial allele then increases in the population over many generations.

- Q3 a) Behavioural adaptation — e.g. hunting in groups/pods.
 Physiological adaptation — reducing heart rate whilst diving. Anatomical adaptation — a thick layer of blubber.
- b) E.g. Hunting in groups/pods helps killer whales to hunt food successfully, increasing their chances of survival. Reducing heart rate whilst diving helps the whales conserve oxygen, so they can last for longer underwater without breathing. This increases their chances of catching prey and so surviving. A thick layer of blubber keeps them warm, so increases their chances of surviving in cold seas. / A thick layer of blubber gives them a streamlined shape, so helps them to move more easily through water to catch their prey, increasing their chances of survival.

The important thing about all adaptations is that they increase an organism's chance of surviving and reproducing successfully.

Page 230 — Fact Recall Questions

- Q1 Random mutations can result in a new allele being formed that is beneficial to the organism. This may then be selected for during natural selection.
- Q2 Not all individuals are as likely to reproduce as each other. There's differential reproductive success in a population — individuals that have an allele that increases their chance of survival are more likely to survive, reproduce and pass on their genes (including the beneficial allele), than individuals with less advantageous alleles. This means that a greater proportion of the next generation inherits the beneficial allele. They, in turn, are more likely to survive, reproduce and pass on their genes. So the frequency of the beneficial allele increases from generation to generation.
- Q3 An adaptation is a feature that increases an organism's chances of survival.
- Q4 Physiological adaptations are processes inside an organism's body that increase its chance of survival.

5. The Effects of Selection

Page 232 — Application Question

- Q1 a) Stabilising selection because the range of masses is being reduced towards the middle of the range.
 b) E.g. light wolves are less likely to survive because they find it more difficult to keep warm in the snow/find it difficult to move through heavy snow. Heavy wolves are also less likely to survive because they sink through the snow more easily/find it harder to hunt for food. Therefore, conditions are most favourable for medium-sized wolves, so the mass of wolves shifted towards the middle of the range.

You weren't expected to know the answer to this question, just to make sensible suggestions as to why smaller and larger wolves were less likely to survive.

Page 232 — Fact Recall Questions

- Q1 Where individuals with alleles for characteristics of an extreme type are more likely to survive and reproduce.
 Q2 Having full antibiotic resistance is a characteristic of an extreme type.

6. Investigating Selection

Page 234 — Application Question

- Q1 a) E.g. sterilised water without antibiotic. It's used to make sure that the water the antibiotic is diluted in is not the reason for any change in turbidity, and hence bacterial growth changes.
- b) Any two from: e.g. regularly disinfect work surfaces to minimise contamination. / Don't put any utensils on the work surface. / Place contaminated utensils in a beaker of disinfectant. / Use sterile equipment and discard safely after use. / Work near a Bunsen flame. / Briefly flame the neck of the container of broth just after it's opened and just before it's closed.
- c) Aseptic techniques are used to prevent contamination of cultures by unwanted microorganisms. This is important because contamination can affect the growth of the microorganism that you're working with. It's also important to avoid contamination with disease-causing microbes that could make you ill.
- d) From 0 to 4 hours, the turbidity of the sample was increasing as the bacteria reproduced and the number in the sample increased. After the addition of the antibiotic at 4 hours, the turbidity remained the same. The antibiotic killed any bacteria present, stopping them reproducing any more and so turbidity stayed at the same level as before the addition of the antibiotic.

Pages 234-235 — Fact Recall Questions

- Q1 E.g. antibiotics, antiseptics, disinfectants
 Q2 distilled water, bacterial culture and nutrients
 Q3 a) A clear patch in a lawn of bacteria where the bacteria can't grow.
 b) It tells you how well an antibiotic/antimicrobial works. The larger the inhibition zone, the more bacteria were inhibited from growing.

Exam-style Questions — pages 236-237

- 1.1 Point 1 (**1 mark**) because the moss's chromosome number goes from diploid to haploid/the chromosome number halves (**1 mark**).
 1.2 Fertilisation (**1 mark**) because the haploid gametes fuse to form a diploid zygote/the chromosome number doubles (**1 mark**).
 1.3 Any one from: e.g. a genetic bottleneck occurred (**1 mark**). / The founder effect meant there were only a small number of alleles in the initial gene pool (**1 mark**). / Through stabilising selection favouring alleles towards the middle of the range and eliminating alleles at the extremes (**1 mark**).
 1.4 E.g. through the introduction of new alleles from gene mutations (**1 mark**). Through new plants, carrying new alleles, being introduced to the population (**1 mark**).
 2.1 Crossing over (**1 mark**)
 2.2 Independent segregation (**1 mark**). When pairs of homologous chromosomes are separated during meiosis I, it's completely random which chromosome from each pair ends up in which daughter cell (**1 mark**). So the daughter cells end up with different combinations of chromosomes, increasing genetic variation in potential offspring (**1 mark**).

- 3 Mitosis produces two daughter cells, whereas meiosis produces four daughter cells (**1 mark**). This is because mitosis only involves one division, whereas meiosis has two divisions (**1 mark**). Mitosis produces cells with the same number of chromosomes as the parent cell, whereas meiosis produces cells with half the number of chromosomes as the parent cell (**1 mark**). This is because mitosis only separates the sister chromatids, whereas meiosis separates the homologous pairs, then the sister chromatids (**1 mark**). In mitosis, daughter cells are genetically identical to each other and to the parent cell, whereas in meiosis daughter cells are genetically different from one another and the parent cell (**1 mark**). This is because there's no pairing or separating of homologous chromosomes in mitosis, and so no crossing over or independent segregation of chromosomes (**1 mark**).
- 4.1 As a result of mutations, some individuals in a population have alleles for producing tetracycline-based antibiotics (**1 mark**). This means they can kill other bacteria in the area, reducing competition for nutrients (**1 mark**). So these bacteria are more likely to survive, reproduce and pass on their alleles to the next generation (**1 mark**). After many generations the frequency of the beneficial allele increases in the population. / After many generations most bacteria in the population will have the alleles to produce tetracycline-based antibiotics (**1 mark**).
- 4.2 Directional selection because that is where individuals with alleles for characteristics of an extreme type, such as antibiotic resistance, are more likely to survive and reproduce (**1 mark**).
- 4.3 Any two from: e.g. they should all be the same breed of cattle. / The cattle should all be the same age. / The cattle should all be kept in the same type of environment. / None of the cattle should have been given any other antibiotics before the experiment started. (**1 mark for each correct answer, up to a maximum of 2 marks**)
- 4.4 To see what percentage of the *E. coli* present in the cows' stomachs were already resistant to tetracycline (**1 mark**).
- 4.5 E.g. tetracycline resistance is present in some of the cattle who do not receive any antibiotics in their feed (**1 mark**) and in cattle that had antibiotics other than tetracycline added to their feed (**1 mark**). Cattle that had both tetracycline and sulfamethazine added to their feed showed the most resistance to tetracycline/more resistance than cattle that had tetracycline alone added to their food (**1 mark**).

Topic 4C — Diversity and Classification

1. Classification of Organisms

Page 240 — Application Questions

- Q1 a) sharks
b) salamanders
c) i) crocodiles
ii) lizards

Q2

| TAXON | |
|---------|----------------|
| Domain | Eukarya |
| Kingdom | Animalia |
| Phylum | Chordata |
| Class | Mammalia |
| Order | Perrisodactyla |
| Family | Equidae |
| Genus | Equus |
| Species | asinus |

Page 240 — Fact Recall Questions

Q1 Based on their evolutionary history.

Q2 Binomial naming system

2. Classification Using Courtship Behaviour

Page 242 — Application Questions

- Q1 That fireflies 4 and 9 belong to the same species.
Fireflies of the same species will have the same pattern of light pulses.
- Q2 6
Different species will have different patterns of light pulses.
- Q3 Distantly. The patterns of light pulses produced by fireflies 1 and 3 are very different.
- Q4 Yes. Fireflies 4 and 9 also start their display with three light pulses so probably belong to the same family.

Page 242 — Fact Recall Questions

- Q1 Behaviour carried out by organisms to attract a mate of the right species.
- Q2 Because it is species specific — only members of the same species will do and respond to that courtship behaviour.

3. Classification Using DNA or Proteins

Pages 244-245 — Application Questions

- Q1 That horses are more closely related to humans than chickens.
- Q2 a) Species A: TCGACGTGGTAATCGAGC
Species B: TCCACGTGTGTAATCGAGT
Species C: ACGCCGAGTGTATGGAGT
b) 3
c) 7
Take your time with questions like this. Once you've got your answer, recount it to make sure it's right.
- d) Species B. There are fewer base differences in the DNA when comparing A and B than A and C.
- e) Species B. There are only 6 base differences between species C and B. This is fewer than for species C and A so species C and B are more closely related.
- Q3 Species B. The higher relative fluorescence indicates that more anti-X antibody bound to cells from species B than to cells from A or C. This suggests that proteins from species B are most similar to those from species X, so B is most closely related to X.

4. Using Gene Technologies to Assess Genetic Diversity

Page 246 — Application Question

- Q1 a) The scientist could compare the DNA base sequences of a gene controlling shell colour or pattern in individuals from a warm climate and individuals from a colder climate. Different alleles of the same gene have slightly different DNA base sequences, so by comparing the base sequences the scientist could determine how many alleles of the gene there are in each population. The scientist could repeat this for all the shell colour and pattern genes.
b) By looking at the frequency of measurable or observable characteristics in a population.

Page 246 — Fact Recall Question

- Q1 Gene technologies can give more accurate estimates of genetic diversity and make comparisons of genetic diversity easier to carry out.

5. Investigating Variation

Page 251 — Application Questions

- Q1 i) The mean wing span is approximately 27 cm for species A and 31 cm for species B. Both curves follow a normal distribution. Species A has a higher standard deviation than species B.
ii) Species A, because it has a higher standard deviation.
b) $(31 - 27) \div 27 \times 100 = 14.8\%$

Make sure you're confident at calculating percentages — they're a common mathsy-type question that examiners like to ask.

- Q2 Work out the mean length of snakes:

$$\bar{x} = (177 + 182 + 190 + 187 + 191) \div 5 = 185.4 \text{ cm}$$

Work out $(x - \bar{x})^2$ for each snake length:

$$A = (177 - 185.4)^2 = (-8.4)^2 = 70.56,$$

$$B = (182 - 185.4)^2 = (-3.4)^2 = 11.56,$$

$$C = (190 - 185.4)^2 = (4.6)^2 = 21.16,$$

$$D = (187 - 185.4)^2 = (1.6)^2 = 2.56,$$

$$E = (191 - 185.4)^2 = (5.6)^2 = 31.36$$

Work out $\Sigma = (x - \bar{x})^2$:

$$70.56 + 11.56 + 21.16 + 2.56 + 31.36 = 137.2$$

Divide it by the number of values minus 1 ($n - 1$):

$$137.2 \div 4 = 34.3$$

Square root it:

$$\sqrt{34.3} = 5.86 \text{ to 3 s.f.}$$

Page 251 — Fact Recall Questions

- Q1 Divide the total of all the values in your data by the number of values in your data.
Q2 Bell shaped
Q3 The spread of the values about the mean. / How much the values in a single sample vary.

6. Biodiversity

Pages 253-254 — Application Questions

- Q1 a) 6

The species richness is just the number of different insect species.

- b) i) Pond A

$$d = \frac{18 \times (18 - 1)}{6 + 20 + 2 + 6 + 0 + 12} = \frac{306}{46} \\ = 6.65 \text{ (3 s.f.)}$$

- ii) Pond B

$$d = \frac{54 \times (54 - 1)}{156 + 20 + 42 + 2 + 306 + 72} = \frac{2862}{598} \\ = 4.79 \text{ (3 s.f.)}$$

If you have to calculate species diversity in the exam, always show your full working out. You may pick up a mark for showing you understand the equation if nothing else.

- c) Pond A. It has a higher diversity of insects, so it will be able to support a higher diversity of birds and amphibians.

- Q2 a) i) Wood

$$d = \frac{101 \times (101 - 1)}{210 + 306 + 272 + 342 + 72 + 56 + 42 + 56 + 0} \\ = \frac{10100}{1356} = 7.45 \text{ (3 s.f.)}$$

- ii) Town

$$d = \frac{41 \times (41 - 1)}{0 + 6 + 2 + 0 + 0 + 2 + 380 + 30 + 20} \\ = \frac{1640}{440} = 3.73 \text{ (3 s.f.)}$$

- b) Comparing the number of species present in a community doesn't take into account the population size of each species. Species that are in a community in very small numbers shouldn't be treated the same as those with bigger populations. For example, the graph shows nine species of tree in the town and eight in the wood. However, eight of the nine tree species in the town are present only in small numbers. The tree species in the wood are all present in higher numbers. Calculating the index of diversity for the wood gives a much higher estimate of biodiversity than simply counting the number of species present.

Page 254 — Fact Recall Questions

- Q1 The variety of living organisms in an area.
Q2 All the populations of different species in a habitat.

7. Agriculture and Biodiversity

Page 257 — Application Question

Q1 a)

| Number of Crop Types | Rank | Diversity Index | Rank | Difference between ranks (d) | d^2 |
|----------------------|------|-----------------|------|------------------------------|-------|
| 1 | 8 | 1.87 | 8 | 0 | 0 |
| 2 | 7 | 2.24 | 7 | 0 | 0 |
| 3 | 6 | 2.71 | 6 | 0 | 0 |
| 4 | 5 | 3.18 | 5 | 0 | 0 |
| 5 | 4 | 4.01 | 3 | 1 | 1 |
| 6 | 3 | 3.59 | 4 | 1 | 1 |
| 7 | 2 | 4.44 | 2 | 0 | 0 |
| 8 | 1 | 4.97 | 1 | 0 | 0 |

$$r_s = 1 - \frac{6 \sum d^2}{n(n^2 - 1)}$$

$$r_s = 1 - \frac{6(0 + 0 + 0 + 0 + 1 + 1 + 0 + 0)}{8(8^2 - 1)}$$

$$r_s = 1 - \frac{6 \times 2}{8 \times 63}$$

$$r_s = 1 - \frac{12}{504}$$

$$r_s = \mathbf{0.976} \text{ (3 s.f.)}$$

- b) Positive
- c) Rejected, because the result is higher than the critical value of 0.738.

Page 258 — Fact Recall Questions

- Q1 a) It increases the area of land available for farming.
 b) Woodland clearance and hedgerow removal reduce diversity by directly removing trees and hedgerow plants. This destroys habitats. Some species also lose their shelter and food source. This means that these species will die or be forced to migrate to another suitable area.
- Q2 Both agriculture and biodiversity are important / provide us with important resources. A balance between agriculture and conservation is needed so we can keep farming and conserve biodiversity.

Exam-style Questions — pages 259-260

- 1.1 E.g. courtship behaviour is species specific (**1 mark**). This reduces the probability of animals mating with different species and producing infertile offspring (**1 mark**).
- 1.2 Length of phrase = 4 seconds
 $60 \div 4 = \mathbf{15 \text{ phrases/minute}} \text{ (**1 mark**)}$
- 1.3 Songbirds A and C produce the same song (**1 mark**). This suggests that they may be the same species (**1 mark**).
- 1.4 E.g. she could calculate the Spearman's rank correlation coefficient (**1 mark**) as this would allow her to determine whether or not there is a correlation between song pitch and body size, as well as the direction/strength of the correlation (**1 mark**).
- 2.1 It is present in all plants so any two species of plant can be compared by looking at RuBisCo (**1 mark**).
- 2.2 Any one from, e.g. by comparing the amino acid sequence (**1 mark**). The more similar the amino acid sequences the more closely related the species are (**1 mark**). / By using immunological comparison (**1 mark**). If two proteins are bound by the same antibody they must be similar and the two species must be closely related (**1 mark**).
- 3.1 The index of diversity takes the number of individuals of each species into account, as well as the overall number of species — unlike species richness (which is just the number of species) (**1 mark**). This gives a more accurate picture of biodiversity because species that are in a community in very small numbers aren't treated the same as those with bigger populations (**1 mark**).
- 3.2 Farm A
- $$d = \frac{47 \times (47 - 1)}{6 + 30 + 72 + 42 + 110 + 110}$$
- $$= \frac{2162}{370} = \mathbf{5.84} \text{ (3 s.f.)}$$
- (2 marks for correct answer, otherwise 1 mark for correct working)**
- Farm B
- $$d = \frac{27 \times (27 - 1)}{132 + 2 + 12 + 30 + 6 + 0}$$
- $$= \frac{702}{182} = \mathbf{3.86} \text{ (3 s.f.)}$$
- (2 marks for correct answer, otherwise 1 mark for correct working)**
- 3.3 Farm A. It has a higher index of diversity (**1 mark**) and using chemical herbicides tends to reduce the number and abundance of species (**1 mark**).
- 3.4 E.g. they could include a sample of hedgerows on farms that do not use biological pesticides (**1 mark**).
- 3.5 E.g. it increases the area of farmland by turning lots of small fields into fewer large fields, which may increase crop production / make it easier to plant/harvest a lot of crops at once (**1 mark**).
- 3.6 Hedgerow removal could reduce insect biodiversity (**1 mark**). It destroys habitats, so insect species could lose their shelter and food sources (**1 mark**). This could kill insects or force them migrate to other areas (**1 mark**).

Topic 5

Topic 5 — A: Photosynthesis and Respiration

1. Photosynthesis, Respiration and Energy

Page 263 — Application Question

Q1 a) 07:30 and 16:30

Anything between 07:20 and 07:40 would be acceptable for the first compensation point. Anything between 16:20 and 16:40 would be OK for the second one.

b) The rate of photosynthesis depends partly on the intensity of light. 07:30 is not long after the Sun has risen. The light intensity has increased to a level where the rate of photosynthesis has increased to match the rate of respiration. 16:30 is not long before the Sun completely sets. The light intensity has decreased to a level where the rate of photosynthesis has decreased to match the rate of respiration.

2. Photosynthesis and the Light-dependent Reaction

Pages 267-268 — Application Questions

Q1 a) i) proton/hydrogen ion/H⁺

ii) Because this forms a proton gradient across the membrane. Protons move down their concentration gradient, into the stroma, via an enzyme called ATP synthase. The energy from this movement combines ADP and inorganic phosphate (P_i) to form ATP.

b) PSII / photosystem II

c) D

d) ATP

Cyclic photophosphorylation doesn't produce any reduced NADP or O₂— just ATP.

Q2 a) photosystem X = photosystem II/PSII
photosystem Y = photosystem I/PSI

b) Light energy absorbed by PSII excites electrons in chlorophyll. This causes the electrons to move to a higher energy level (i.e. they have more energy).

c) To transport protons into the thylakoid.

In this way a proton gradient is formed across the thylakoid membrane. As the protons move down this concentration gradient, back into the stroma, ATP is formed by ATP synthase.

d) The electrons are transferred to NADP, along with a proton from the stroma, to form reduced NADP.

Page 268 — Fact Recall Questions

Q1 a) Coloured substances that absorb the light energy needed for photosynthesis.

b) E.g. chlorophyll a / chlorophyll b / carotene.

Q2 hydrogen

Q3 the thylakoid membranes

Q4 Light energy excites the electrons in the chlorophyll molecule, giving them more energy, which eventually causes them to be released from the chlorophyll. The chlorophyll is left as a positively charged ion.

Q5 ATP and reduced NADP

Q6 The process of adding phosphate to a molecule using light.

Q7 A chain of proteins down which excited electrons flow.

Q8 a) protons, electrons and oxygen

b) To replace excited electrons in PSII.

Q9 This energy is used to transport protons into the thylakoid so that the thylakoid has a higher concentration of protons than the stroma. This forms a proton gradient across the membrane. Protons move down their concentration gradient, into the stroma, via an enzyme called ATP synthase. The energy from this movement combines ADP and inorganic phosphate (P_i) to form ATP.

Q10 a) ATP, reduced NADP and oxygen

b) ATP

3. Photosynthesis and the Light-independent Reaction

Page 271 — Application Questions

Q1 Increasing the speed of rubisco could increase the production rate of glyceral 3-phosphate from ribulose bisphosphate and carbon dioxide, as rubisco catalyses this reaction. The increased production rate of glyceral 3-phosphate would increase the production rate of triose phosphate, which in turn could be converted into organic substances such as glucose more quickly.

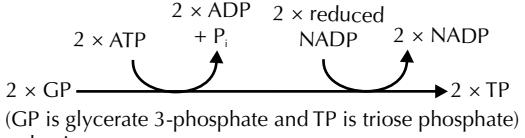
Q2 The rate of the light-independent reaction would slow down, because the amount of ribulose bisphosphate that could be regenerated in the Calvin cycle would decrease.

Page 271 — Fact Recall Questions

Q1 a) ribulose bisphosphate (RuBP)

b) glyceral 3-phosphate (GP)

Q2 a)



b) reduction

Q3 In the Calvin cycle ATP is needed for the reduction of glyceral 3-phosphate (GP) to triose phosphate (TP). It's also needed for the regeneration of ribulose bisphosphate (RuBP) from triose phosphate.

Q4 five

Q5 a) six

Six turns of the Calvin cycle produces 12 molecules of triose phosphate (TP). Ten of these molecules (5 out of every 6) are used to make ribulose bisphosphate (RuBP) and two are used to make one hexose sugar.

b) 18

Six turns of cycle × 3 ATP molecules per turn = 18 ATP

c) 12

Six turns of cycle × 2 reduced NADP molecules per turn = 12 reduced NADP

Q6 a) Two triose phosphate molecules are joined together to produce a hexose sugar. Large carbohydrates are then made by joining the hexose sugars together.

b) Lipids are made from glycerol and fatty acids. Glycerol is synthesised from triose phosphate, while fatty acids are made from glyceral 3-phosphate.

4. Limiting Factors in Photosynthesis

Page 275 — Application Question

- Q1 a) Increased irradiance results in an increased rate of photosynthesis, which means that more CO₂ is needed, so uptake increases.
- b) Because the CO₂ concentration becomes the limiting factor for photosynthesis. As the rate of photosynthesis is no longer increasing, the rate of CO₂ uptake remains the same.
- c) E.g. the data suggests that increasing the atmospheric CO₂ concentration increases CO₂ uptake. This suggests that the rate of photosynthesis also increases. An increased rate of photosynthesis would lead to an increase in the production of sugars/glucose for respiration and so an increase in ATP production. In turn, this could lead to an increased growth rate — but there is nothing in the data to suggest that this would improve the yield of tomatoes (e.g. the plants themselves may grow bigger, but the number of tomatoes they produce might stay the same). This data was also collected under laboratory conditions, in which the temperature was kept constant — this may be harder to do in a glasshouse and using a paraffin heater, which may mean that these results wouldn't apply in glasshouse conditions.

5. Photosynthesis Experiments

Page 278 — Application Question

- Q1 a) Different pigments will spend different amounts of time in the mobile phase, so they will travel different distances up the plate in the same amount of time, which separates them out.
- b) R_f value = $\frac{\text{distance travelled by spot}}{\text{distance travelled by solvent}}$
= 3.47 cm ÷ 9.00 cm = **0.386** (3 s.f.)

Page 279 — Fact Recall Questions

- Q1 In all chromatography, a mobile phase (e.g. a liquid solvent) moves over a stationary phase (e.g. chromatography paper or a TLC plate). The components in the mixture spend different amounts of time in the mobile phase and the stationary phase. The components that spend longer in the mobile phase travel faster or further. The time spent in the different phases is what separates out the components of the mixture.
- Q2 DCPIP changes from blue to colourless when it gets reduced, indicating that a redox reaction has taken place.
- Q3 E.g. the leaves could be cut up into pieces, their stalks removed, and then ground up using a pestle and mortar with some cold isolation solution. The liquid could then be filtered into a beaker through muslin cloth and transferred to centrifuge tubes. These tubes would be centrifuged at high speed, causing the chloroplasts to gather in pellets at the bottom of each tube. The liquid above the pellets could then be removed and the pellets re-suspended in fresh isolation solution to form the chloroplast extract.

6. Aerobic and Anaerobic Respiration

Page 282 — Application Questions

- Q1 a) It catalyses the phosphorylation of glucose to glucose phosphate, using a phosphate from ATP.
b) E.g. it may help to stop the over-production of glucose phosphate.
- Q2 a) i) lactate/lactic acid
ii) pyruvate
iii) ethanol
b) i) A
ii) B
iii) A

Page 282 — Fact Recall Questions

- Q1 ATP is used to phosphorylate glucose, making triose phosphate.
- Q2 In the oxidation of triose phosphate to pyruvate, NAD collects the hydrogen ions from triose phosphate, forming reduced NAD.
- Q3 10
Two molecules of pyruvate are made for every molecule of glucose that enters glycolysis.
- Q4 It is used in glycolysis (to collect the hydrogen ions lost from triose phosphate in the production of pyruvate).
- Q5 lactate/lactic acid

7. Aerobic Respiration — The Mitochondrial Reactions

Page 284 — Application Questions

- Q1 a) oxaloacetate = 4C, citrate = 6C
b) Decarboxylation and dehydrogenation occur, producing one molecule of reduced FAD and two of reduced NAD. ATP is produced by substrate level phosphorylation.
- Q2 24
Two molecules of carbon dioxide are produced per turn of the Krebs cycle and the Krebs cycle turns twice for each molecule of glucose. So for one molecule of glucose four molecules of carbon dioxide are produced. Therefore if six molecules of glucose were respired, 24 (6 × 4) molecules of carbon dioxide would be produced in the Krebs cycle.
- Q3 Acetyl coenzyme A can enter the Krebs cycle, leading to the formation of reduced coenzymes, which are then used in oxidative phosphorylation.

Page 287 — Application Questions

- Q1 a) Carrier 1 will be in a reduced state because it has received electrons from reduced NAD but can't pass them on. Carrier 3 will be in an oxidised state because it has passed its electrons onto oxygen, but hasn't received any more from carrier 2.
- If a substance gains electrons it is reduced. If a substance loses electrons it is oxidised.
- b) Antimycin A inhibits carrier 2 and so stops electrons moving down the electron transport chain. This means no more energy will be lost from electrons moving down the chain, so H⁺ ions will not be transported across the inner mitochondrial membrane and the electrochemical gradient across the membrane won't be maintained. This means the synthesis of ATP by ATP synthase will stop. If a fish can't produce ATP it will die as energy from ATP is needed to fuel all biological processes.
- Q2 The fact that ATP synthesis stops when DCC is added suggests that the movement of protons through the ATP synthase is essential for ATP production. This supports the chemiosmotic theory because it suggests that the proton gradient is being used to synthesise ATP.

Page 287 — Fact Recall Questions

- Q1 a) Pyruvate is decarboxylated — one carbon atom is removed from pyruvate in the form of carbon dioxide. Then NAD is reduced — it collects hydrogen from pyruvate. Pyruvate becomes oxidised and acetate is formed.
- b) It combines with acetate to form acetyl coenzyme A.
- c) Acetyl coenzyme A enters the Krebs cycle. Reduced NAD is used in oxidative phosphorylation. Carbon dioxide is released as a waste product.
- Q2 a) Two molecules of CO₂ are released — one CO₂ is released from the conversion of citrate to a 5-carbon compound and the other CO₂ is released from the conversion of the 5-carbon compound to oxaloacetate.
- b) one
- Q3 substrate-level phosphorylation
- Q4 a) It is reused in the link reaction.
- b) It is regenerated for use in the next Krebs cycle.
- Q5 They lose energy.
- Q6 oxygen
- Q7 E.g. the conversion of pyruvate to acetate in the link reaction. / The conversion of citrate to the 5-carbon compound in the Krebs cycle. / The conversion of the 5-carbon compound to oxaloacetate in the Krebs cycle. Every time CO₂ is lost in a reaction, decarboxylation is happening.
- Q8

| Substance | Glycolysis | Link reaction | Krebs cycle | Oxidative phosphorylation |
|-----------------|------------|---------------|-------------|---------------------------|
| ATP | X | | X | X |
| reduced NAD | X | X | X | |
| reduced FAD | | | X | |
| CO ₂ | | X | X | |

8. Respiration Experiments

Page 289 — Application Question

- Q1 a) E.g. the scientist could have set up a test tube containing a known volume and concentration of substrate (e.g. glucose) solution and a buffer solution at specific pH. She could then have added a known mass of dried yeast of species A to the tube and stirred until the yeast dissolved. Next, she could have sealed the test tube with a bung and attached it via a tube to a gas syringe in order to catch the CO₂ produced by the respiring yeast. At regular intervals (e.g. every minute) for a set amount of time (e.g. 10 minutes), the scientist could have recorded the volume of gas present in the gas syringe. By repeating the experiment (e.g. three times) at this pH, she could then have calculated the mean rate of CO₂ production at this pH. She could then have repeated the experiment at a range of pH levels by using buffer solutions of different pH levels. She could have done the same thing for species B.
- b) Respiration is a series of reactions controlled by enzymes. The enzymes used in respiration by the different species of yeast may have different optimum pH levels, at which they are able to catalyse the reactions most effectively.
- c) Mean rate of CO₂ production of species A at pH 5.5 = 1.75 cm³ min⁻¹
- Anything between 1.7 and 1.8 cm³ would be acceptable here.
- Mean rate of CO₂ production of species B at pH 5.5 = 3.75 cm³ min⁻¹
- Anything between 3.7 and 3.8 cm³ would be acceptable here.
- Percentage change in rate from species A to species B = $((1.75 - 3.75) \div 1.75) \times 100 = 114\% \text{ faster}$
- Your final answer may differ a little depending on what you got for the mean rates of CO₂ production for the two species.
- d) Boiled yeast won't respire as the boiling will have killed it. Therefore, it acts as a negative control to show that the CO₂ production is a result of the respiring yeast and not any other reactions that may be happening in the tube.

Page 291 — Fact Recall Questions

- Q1 To stop oxygen getting into the yeast solution, forcing the yeast to respire anaerobically.
- Q2 Prepare and treat a test tube in the same way as the others in the investigation, but do not put any yeast in it.
- Q3 10 g
- You know the answer here is 10 g because the mass of the peas and the mass of the glass beads in the control tube have to be the same.

Exam-style Questions — pages 293-294

- 1.1 E.g. if the mitochondria can't produce proteins, they won't be able to produce the enzymes needed to make ATP (e.g. ATP synthase) (**1 mark**). They won't be able to produce proteins that form part of the electron transport chain, which is needed to make ATP (**1 mark**).
- 1.2 Because glycolysis takes place in the cytoplasm of the cell (**1 mark**).
Because glycolysis takes place in the cytoplasm and not the mitochondria, it doesn't matter whether you have functioning mitochondria or not — glycolysis can still happen.
- 1.3 Glucose is phosphorylated using ATP to create glucose phosphate (**1 mark**). Glucose phosphate is phosphorylated using ATP to form hexose bisphosphate/a six carbon intermediate (**1 mark**), which is split to form two molecules of triose phosphate (**1 mark**).
- 1.4 In the oxidation of triose phosphate to pyruvate, NAD collects the hydrogen ions from triose phosphate (**1 mark**), forming reduced NAD (**1 mark**).
- 2.1 A sample of the solution could have been placed in a cuvette (**1 mark**) and put in a colorimeter, a machine that measures absorbance (**1 mark**).
- 2.2 As DCPIP is reduced and loses its blue colour, the absorbance of the solution will decrease (**1 mark**). When photosynthesis occurs at a faster rate, DCPIP will be reduced at a faster rate (**1 mark**), and absorbance will decrease faster as a result (**1 mark**).
- 2.3 Because the high temperature has denatured the enzymes involved in photosynthesis, meaning that photosynthesis cannot occur (**1 mark**). As a result, the colour of the DCPIP won't change and neither will the absorbance (**1 mark**).
- 3.1 The oxidation of triose phosphate to pyruvate produces one molecule of reduced NAD (**1 mark**). The conversion of pyruvate to acetate produces one molecule of reduced NAD (**1 mark**). The conversion of citrate to a 5-carbon compound in the Krebs cycle produces one molecule of reduced NAD (**1 mark**). The conversion of this 5-carbon compound to oxaloacetate produces another two molecules of reduced NAD (**1 mark**) and one molecule of reduced FAD (**1 mark**).
- 3.2 The electrons move down the electron transport chain, losing energy at each electron carrier (**1 mark**). Finally they are passed onto oxygen as it is the final electron acceptor (**1 mark**).
- 3.3 There would be no electrochemical gradient produced across the inner mitochondrial membrane (**1 mark**). This means there would be no movement of ions across the mitochondrial membrane to drive ATP synthase (**1 mark**) so no ATP would be made (**1 mark**). The cells would only get ATP from anaerobic respiration (**1 mark**).
Even though H⁺ ions will still be pumped across the inner mitochondrial membrane into the intermembrane space, the uncoupler will be moving them back into the matrix at the same time — so no gradient would be produced.
- 4.1 The student hasn't taken into account the amount of oxygen that the plant has used for respiration (**1 mark**), so less oxygen will be released by the plant than it has actually produced during photosynthesis (**1 mark**).
- 4.2 The limiting factor in experiment 2 must be temperature because the graph for experiment 3 levels off at a higher point (**1 mark**) but experiment 3 had the same light intensity and CO₂ concentration as experiment 2 (**1 mark**).

- 4.3 The level of RuBP will have increased because there would have been less CO₂ to combine with RuBP to form GP (**1 mark**). The level of TP will have decreased because less GP would have been made and so less converted to TP (**1 mark**). As TP was made into useful organic substances this will have decreased the level of TP further (**1 mark**). If you get a question like this in the exam, make sure you think of the substances before the reactant in the cycle as well as those that come after it.

Topic 5 — B: Energy Transfer and Nutrient Cycles

1. Energy Transfer in Ecosystems

Page 298 — Application Questions

- Q1 a) He could have dried out the sample he took in an oven set at a low temperature, checking the weight every day until it became constant. At this point, all the water would have been removed and this would be the dry mass of the sample.
b) It will give him an estimate of the chemical energy stored in the wheat.
- Q2 a) $N = I - (F + R)$
 $N = 57\ 153 - (34\ 292 + 17\ 000)$
 $N = \mathbf{5861\ kJ\ m^{-2}\ yr^{-1}}$
- b) % efficiency of energy transfer =
(net productivity of trophic level ÷ net productivity of previous trophic level) × 100
 $(627 \div 5861) \times 100 = \mathbf{10.7\%}$
- Q3 a) $NPP = GPP - R$. This can be rearranged to give:
 $GPP = NPP + R$, so
 $GPP = 31\ 023 + 15\ 604$
 $= \mathbf{46\ 627\ kJ\ m^{-2}\ yr^{-1}}$
- b) $N = I - (F + R)$. This can be rearranged to give:
 $R = I - F - N$, so
 $R = 5983 - 2729 - 2073$
 $= \mathbf{1181\ kJ\ m^{-2}\ yr^{-1}}$

Remember, N is the net productivity of the small fish, I is the amount of energy the small fish ingest from food, F is the amount of energy lost in faeces and urine, and R is the respiratory loss of the fish. You're given the numbers you need for I and F in the question — you just have to find N from the diagram, and rearrange the formula to find R . Double check your answer by plugging the values for I , F , and R back into the equation for N . If you've calculated R correctly, you should come up with

$$N = 2073\ KJ\ m^{-2}\ yr^{-1}.$$

- c) $N = I - (F + R)$. This can be rearranged to give:
 $F = I - R - N$, so
 $F = 2073 - 879 - 119$
 $= \mathbf{1075\ kJ\ m^{-2}\ yr^{-1}}$

Again, rearrange the formula and put in the values you know. R is given in the question, N is shown on the diagram and I is also on the diagram — it's the net productivity of the small fish.

- d) Any two from: e.g. because some parts of the small fish aren't eaten so the energy isn't taken in. / Because some parts of the small fish are indigestible and so are egested as faeces. / Because some of the energy is lost to the environment through respiration or excretion of urine.
- e) % efficiency of energy transfer =
 (net productivity of trophic level ÷ net productivity of previous trophic level) × 100
 Between plant plankton and animal plankton =
 $(8105 \div 31023) \times 100 = 26.1\%$
 Between animal plankton and small fish =
 $(2073 \div 8105) \times 100 = 25.6\%$
 Between small fish and large fish =
 $(119 \div 2073) \times 100 = 5.7\%$

Page 298 — Fact Recall Questions

- Q1 The mass of living material in the plant. / The chemical energy stored in the plant.
- Q2 The total amount of chemical energy converted from light energy by plants, in a given area.
- Q3 The energy lost to the environment as heat when organisms respire.
- Q4 a) The energy stored in the consumers' biomass. It is also the energy available to organisms at the next trophic level.
 b) $N = I - (F + R)$

2. Farming Practices and Production

Page 301 — Application Question

- Q1 a) rate = $\frac{\text{change in } y}{\text{change in } x}$
 $= \frac{57 - 47}{22 - 18}$
 $= 2.5 \text{ kg week}^{-1}$
- b) Breed 1: $64 \div 100 \times 82 = 52 \text{ kg}$ (2 s.f.)
 Breed 2: $73 \div 100 \times 76 = 55 \text{ kg}$ (2 s.f.)
 Breed 3: $66 \div 100 \times 57 = 38 \text{ kg}$ (2 s.f.)
Breed 2 would produce the most meat at 22 weeks.
- c) E.g. using antibiotics may mean that the pigs use less energy fighting diseases so they can use more energy to grow, increasing their net production.
- d) Any one from: e.g. keep the pigs in pens to restrict their movement. This will reduce the energy lost through respiration. / Keep the pigs indoors, so that they are kept warm. This will reduce the energy lost through generating body heat.

Page 301 — Fact Recall Questions

- Q1 Simple lines of energy transfer through an ecosystem.
- Q2 Lots of overlapping food chains in an ecosystem.
- Q3 Simplifying the food web gets rid of food chains that don't involve humans/involving pests. This means that less energy is transferred to the pests, increasing the efficiency of energy transfer to humans.
- Q4 E.g. pesticides / insecticides / herbicides / biological agents.

3. Nutrient Cycles in Natural Ecosystems

Pages 304-305 — Application Questions

- Q1 a) $\frac{(31.5 + 107)}{(5.4 + 107 + 31.5 + 86)} \times 100$
 $= 60\%$ (2 s.f.)
- b) $\frac{120 - 107}{120} \times 100$
 $= 11\%$ (2 s.f.)
- c) E.g. more land was being cultivated in the 1990s than in 1860, so there was less uncultivated land.
- d) E.g. through the manufacture of fertilisers/increased use of fertilisers.
- Q2 It might decrease because waterlogged soils create anaerobic conditions. This means that denitrifying bacteria convert nitrates in the soil back into nitrogen gas, which the plants can't assimilate without nitrogen fixation.
- Q3 a) Figure 2 because phosphorus is not cycled through the atmosphere, unlike nitrogen.
 Don't let the fact that both the figures show the ocean as part of the cycle throw you. You know that nitrogen is needed for plant growth and that aquatic ecosystems also involve plants.
- b) As phosphate ions dissolved in water.
- c) Ammonification.
- d) Phosphate ions dissolved in the oceans are assimilated by aquatic producers (such as algae).
- e) Through the death and decomposition of organisms and thorough the breakdown of compounds in faeces and urine.

Page 305 — Fact Recall Questions

- Q1 a) Fungi, bacteria.
 b) They secrete enzymes and digest their food externally, then absorb the nutrients they need.
- Q2 a) Mycorrhizae are symbiotic relationships between fungi and the roots of plants.
 b) The fungi have long thin stands called hyphae which connect to the plant's roots. The hyphae greatly increase the surface area of the plant's root system, allowing the plant to absorb more water and mineral ions.
- Q3 nitrogen fixation, nitrification, denitrification and ammonification
- Q4 Saprobionts are involved in breaking down the organic compounds when plants and animals die, releasing phosphate ions into the soil for assimilation by plants. They also release the phosphate ions from urine and faeces.
- Q5 a) The waste produced by sea birds.
 b) Because it returns a significant amount of phosphate ions to the soil from the oceans.

4. Fertilisers and Eutrophication

Pages 307-308 — Application Questions

- Q1 a) 100 m and 800 m, because these sites are where the nitrate concentration increases sharply.
- b) i) $37 - 31 = 6 \text{ mg l}^{-1}$
 $(6 \div 31) \times 100 = 19.4\%$
- ii) E.g. the second farm has more land on which crops are grown so more nitrate fertiliser is leached into the river. / The second farm uses a higher concentration of nitrate fertiliser on its land. / The second farm uses a chemical fertiliser whilst the first farm uses a natural/organic fertiliser. / The second farm applies more nitrate fertiliser than the crops can use. / The second farm applies fertiliser before heavy rain.
- There are lots of possible reasons why the water next to the second farm has a higher nitrogen concentration than the water next to the first farm — you just need to give a sensible answer.
- Q2 The nitrate concentration and algal content of the control river remain constant at 7 mg l^{-1} and $10 \text{ thousand cells cm}^{-3}$. This indicates that the nitrate concentration and algal growth on rivers A and B were affected by the two farms and not due to any other environmental variable.
- Q3 a) There is a correlation between the nitrate concentration of river A and its algal content. Shortly after the nitrate concentration increases in river A, the algal content increases too.
- b) Nitrates leached from fertilised fields stimulate the growth of algae in rivers.
- Q4 Large amounts of algae may block light from reaching the plants below. Eventually the plants might die because they're unable to photosynthesise enough. Bacteria would then feed on the dead plant matter. The increased numbers of bacteria would reduce the oxygen concentration in the water by carrying out aerobic respiration. This could reduce the number of fish and other aquatic organisms at these locations because there isn't enough dissolved oxygen in the water.

Page 308 — Fact Recall Questions

- Q1 Crops take in mineral ions from the soil as they grow. When crops are harvested, they're removed from the field where they're grown rather than being allowed to die and decompose there. This means the mineral ions that they contain (e.g. phosphates and nitrates) are not returned to the soil by decomposers in the nitrogen or phosphorus cycles.
- Q2 E.g. manure / composted vegetables / crop residues / sewage sludge.
- Q3 leaching
- Q4 eutrophication

Exam-style Questions — pages 310-311

- 1.1 The Sun (**1 mark**). Photosynthesis (**1 mark**).
- 1.2 $N = I - (F + R)$
 $N = 2619 - (1571 + 785)$
 $= 263 \text{ kJ m}^{-2} \text{ yr}^{-1}$ (**1 mark**)
- 1.3 percentage efficiency of energy transfer =
(net productivity of trophic level ÷ net productivity of previous trophic level) × 100
Between the producer and primary consumer 1 =
 $(2619 \div 38750) \times 100 = 6.76\%$
Between the producer and primary consumer 2 =
 $(1265 \div 38750) \times 100 = 3.26\%$
 $6.76 - 3.26 = 3.5\%$
(2 marks for the correct answer, or 1 mark for either 6.76% or 3.26%)
- 1.4 The saprobiots feed on the remains of the dead organisms and on their waste products, breaking them down (**1 mark**). This allows important chemical elements in the remains and waste to be recycled (**1 mark**).
- 2.1 Mycorrhizae are symbiotic relationships that form between some fungi and plant roots (**1 mark**).
- 2.2 They should have controlled, any two from: e.g. the mineral content of the soil. / The mass of soil used. / The volume of water added to the soil. / The temperature that the two groups of seedlings were kept at. / The light intensity that the two groups of seedlings were exposed to.
(Any two correct answers for 1 mark)
- 2.3 Mean mass of seedlings on day 30 = 5 g.
Mean mass of seedlings on day 60 = 10 g.
 $(10 - 5) \div 30 = 0.2 \text{ g day}^{-1}$ (1 s.f.) (**1 mark**)
- 2.4 The fungi used to inoculate the mycorrhizal culture group increased the surface area of the seedlings' root systems compared to those in the control group (**1 mark**). This increased the mycorrhizal group seedlings' uptake of water/important mineral ions compared to the seedlings in the control group (**1 mark**). This increased the growth of the seedlings in the mycorrhizal group (**1 mark**).
- 3.1 A field of potato crops with greenfly infestation that wasn't treated with any form of pest control (**1 mark**).
- 3.2 Field D is the negative control field because the consistently high numbers of greenfly reduce the amount of energy available to the crops for growth (**1 mark**), which means the crops are less efficient at converting energy so it will have the lowest net primary production (**1 mark**). Field A has been treated by an integrated system because at the end of the study it has the lowest numbers of greenfly (**1 mark**), which means the crops have lost the least energy and biomass, so net primary production will be the highest (**1 mark**).
- 3.3 E.g. take samples of the crop before and after the study period (**1 mark**). Dry the samples out in an oven, checking the weight each day until they become constant (**1 mark**). This gives the dry mass of each sample. Use the difference in mass between the two samples (**1 mark**) divided by the length of the study period, to determine the rate at which biomass has been added (**1 mark**).

Topic 6

Topic 6 — A: Stimuli and Responses

1. Survival and Response

Page 313 — Application Question

- Q1 a) tactic/taxis
b) An environment higher in oxygen is a more favourable environment for them to be in.

Page 313 — Fact Recall Questions

- Q1 To increase their chances of survival.
Q2 Directional movement in response to a stimulus.
Q3 Non-directional (random) movement in response to a stimulus.

2. Nervous Communication

Page 316 — Application Questions

- Q1 a) Any one from, e.g. the response would be slower / the response would be voluntary.
b) Stimulus — light tap/touch.
Effector — quadriceps muscle.
c) i) The knee-jerk reflex doesn't involve a relay neurone in the spinal cord. / There are usually three neurones involved in a simple reflex.
ii) E.g. the quadriceps muscle may not contract/there may be no response. If the spinal cord is damaged then the sensory neurone may not be able to transmit nervous impulses to the motor neurone / the motor neurone may not be able to transmit nervous impulses to the leg muscle.
- Q2 a) The nociceptors detect the stimulus and impulses are passed to a sensory neurone. This passes the electrical impulses to a relay neurone in the spinal cord/CNS which carries the impulse to a motor neurone. The motor neurone carries impulses to an effector (e.g. a biceps muscle).
b) Particular receptors are specific to a particular stimulus. This means that it's possible that while their pain receptors aren't functional (so pain isn't felt), their touch receptors are functional allowing light touches to be felt.
c) It helps to protect the body by reacting to situations/ environments that could cause the body harm.

Page 316 — Fact Recall Questions

- Q1 To detect stimuli.
Q2 Muscle cells / cells found in glands.
Q3 a) Transmits electrical impulses from receptors to the central nervous system (CNS).
b) Transmits electrical impulses from the CNS to effectors.
c) Transmits electrical impulses between sensory neurones and motor neurones.
Q4 a) Receptor cells detect a stimulus. Sensory neurones transmit electrical impulses from the receptors to the CNS. The CNS processes the information and sends impulses along motor neurones to effectors, which respond.
This question asks about a voluntary response, so make sure that your answer includes the CNS processing the information.
b) CNS
Q5 The response is localised because neurotransmitters are secreted directly onto cells. The response is short-lived because neurotransmitters are quickly removed once they have done their job.
Q6 The pathway of communication goes through the spinal cord but not through conscious parts of the brain, so the response is automatic.
Q7 Because they're so rapid.

3. Responses in Plants

Page 319 — Application Question

- Q1 a) positive
The shoot is bending towards the stimulus.
b) Y because this is where cell elongation is taking place, causing the shoot to bend towards the opposite side.

Page 319 — Fact Recall Questions

- Q1 phototropism
Q2 They grow in the opposite direction to the force of gravity.
Q3 The growing regions of the plant / shoot and root tips.
Q4 They stimulate growth by cell elongation.
Q5 IAA is an auxin that's produced in the tips of shoots and roots in flowering plants.
Q6 By diffusion and active transport over short distances, and via the phloem over long distances.
Q7 a) IAA concentration increases on the shaded side of the shoot. This means the cells on the shaded part of the shoot grow faster than the cells most exposed to light. This pattern of growth causes the shoot to bend towards the light.
b) IAA concentration increases on the underside of roots. This means the cells on the underside of the root don't grow as quickly as the cells on the upper-side. This pattern of growth causes the root to grow downwards in the same direction as gravity.

4. Receptors

Page 321 — Application Questions

- Q1 a) threshold level
b) B, because its generator potential reaches -60mV/the threshold level.
c) Approximately -87.5 mV (accept any value between -87 mV and -88 mV)
- Make sure you always read the axes carefully — especially on graphs to do with potential differences across cell membranes, because they nearly always involve negative numbers.
- Q2 E.g. pressure from touch would normally deform the stretch-mediated sodium ion channels in Pacinian corpuscles. However, by blocking sodium ion channels the drug would stop sodium ions from diffusing into the cell and generating an action potential. This would mean the person wouldn't be able to perceive that they were being touched.

Page 323 — Fact Recall Questions

- Q1 When a stimulus is detected, the cell membrane is excited and becomes more permeable, allowing more ions to move in and out of the cell. This alters the potential difference across the cell membrane and therefore produces a generator potential.
- Q2 mechanical
- Q3 A Pacinian corpuscle contains the end of a sensory neurone. The sensory nerve ending is wrapped in layers of connective tissue called lamellae.
- Q4 When a Pacinian corpuscle is stimulated the lamellae are deformed and press on the sensory nerve ending. This causes deformation of stretch-mediated sodium ion channels in the sensory neurone's cell membrane. The sodium ion channels open and sodium ions diffuse into the cell, creating a generator potential. If the generator potential reaches the threshold, it triggers an action potential.
- Q5 Cones are close together and each cone joins one bipolar neurone. So when light from two points that are close together hits two cones, an action potential from each cone goes to the brain. This means that the light can be distinguished as coming from two separate points. This doesn't happen in rods because many rods join the same bipolar neurone, which means light from two points close together can't be told apart.
- Q6 Any three from: e.g. rods are found mainly in the peripheral parts of the retina and cones are mainly found packed together in the fovea. / Rods only give information in black and white but cones give information in colour. / Rods are very sensitive to light but cones are less sensitive. / Many rods join one bipolar neurone, but only one cone joins one bipolar neurone.

5. Control of Heart Rate

Page 326 — Application Question

- Q1 The chemoreceptors in a person with anaemia will detect low oxygen levels in the blood. The chemoreceptors will send impulses along sensory neurones to the medulla, which will send impulses along sympathetic neurones. These neurones will secrete noradrenaline, which will bind to receptors on the sinoatrial node/SAN and cause the heart rate to increase in an attempt to increase oxygen levels in the blood.

Page 326 — Fact Recall Questions

- Q1 a) To control unconscious activities of the body.
b) The sympathetic nervous system and the parasympathetic nervous system.
- Q2 That it can contract and relax without receiving signals from nerves.
- Q3 To act as a pacemaker, setting the rhythm of the heartbeat by sending out waves of electrical activity to the atrial walls.
- Q4 medulla (oblongata)
- Q5 a) baroreceptor/pressure receptor
b) aorta and carotid arteries

Exam-style Questions — pages 328-329

- 1.1 phototropism (**1 mark**)
1.2 The seedling should have been from a Goosegrass plant and potted in soil from the same source (**1 mark**). There should have been no lamp/light from any direction present (**1 mark**).
1.3 E.g. to make sure that only the variable being tested (light intensity) was changing (**1 mark**). / To keep all variables other than light intensity the same (**1 mark**).
1.4 Seedling A will be bent to the right because it will have grown towards the light (**1 mark**). Seedling B will have grown straight up because the rotation of the seedling means that the light is not continuously coming from one direction (**1 mark**). Seedling C will be bent towards the right but may have a kink in, so that it is not a smooth bend because it will have grown to the right for five days, then to the left for five days and to the right again for the last five days, as the position of the light source has been changed (**1 mark**).
1.5 IAA/auxins would become most concentrated in the shaded parts of the plant/shoots (**1 mark**). This would mean that the shaded parts of the shoot would grow faster/elongate more than the parts exposed to light (**1 mark**). This uneven growth would lead to the shoots bending towards the light (**1 mark**).
2.1 Light enters the eye, hits the photoreceptors/cones and is absorbed by light-sensitive optical pigments (**1 mark**). Light bleaches the pigments, causing a chemical change and altering the membrane permeability to sodium ions (**1 mark**). A generator potential is created and if it reaches the threshold an action potential is sent along a bipolar neurone (**1 mark**). These connect photoreceptors to the optic nerve, which takes impulses to the brain where the information is interpreted as colour (**1 mark**).
2.2 E.g. in low light conditions only rods are sufficiently light sensitive to trigger action potentials and send information to the brain (**1 mark**). This is because many rods join one bipolar neurone, so many weak generator potentials can combine to reach the threshold and trigger an action potential in low light (**1 mark**). However, bright light is required to trigger an action potential in a cone (**1 mark**) because each cone joins one neurone so it takes more light to reach the threshold and trigger an action potential (**1 mark**). In protanopia only the cones are affected so the ability to see in low light is not affected (**1 mark**).
2.3 E.g. bright light entering the eye could cause damage to it/the photoreceptors/retina (**1 mark**). As the reflex to contract the pupil takes place quickly this reduces the risk of the eye being damaged (**1 mark**).

- 2.4 The photoreceptors detect the bright light and send an impulse down a sensory neurone to the CNS (**1 mark**). In the CNS a relay neurone carries the impulse to a motor neurone (**1 mark**), which carries the impulse to muscles in the eye causing them to contract and the pupil to narrow/reduce in size (**1 mark**).
- 3.1 The delay gives time for the atria to fully contract and empty before the ventricles contract (**1 mark**).
- 3.2 It prevents the waves of electrical activity from being passed directly from the atria to the ventricles (**1 mark**).
- 3.3 The AVN passes the waves of electrical activity onto the bundle of His (**1 mark**). The bundle of His conducts the waves of electrical activity between the ventricles down to the apex/bottom of the heart to the Purkyne tissue (**1 mark**). The Purkyne tissue carries the waves of electrical activity into the muscular walls of the right and left ventricles causing them to contract simultaneously from the bottom up, which results in blood being pumped out of the heart (**1 mark**).
- 3.4 Noradrenaline is a neurotransmitter which binds to receptors on the sinoatrial node, causing the heart rate to speed up (**1 mark**). Beta-blockers therefore stop the heart rate being increased in response to low blood pressure or low blood oxygen levels (**1 mark**), so beta-blockers could be contributing to the slow heart rate/AV block symptoms seen in the patient (**1 mark**).
- 3.5 E.g. the delay in passage of electrical activity through the AVN caused by the second-degree AV block can result in a decreased heart rate (**1 mark**). This is because of the increased time taken for electrical activity to reach the ventricles and cause their contraction/the heart to beat (**1 mark**). This slow heart rate can result in low oxygen levels in the blood, which could cause dizziness and fainting (**1 mark**).
- 3.6 The aorta, carotid arteries and medulla (**1 mark**).

Topic 6 — B: Nervous Coordination

1. Neurones

Page 334 — Application Questions

- Q1 A — The neurone is stimulated.
 B — Depolarisation / Lots of sodium ion channels are open and lots of sodium ions are diffusing into the neurone.
 C — The sodium ion channels are closed and the potassium ion channels are open.
- Q2 -40 mV
- Q3 -60 mV
 Remember to always include units in your answer when they're given on the graph.
- Q4 a) At a potential difference of $+40\text{ mV}$ the sodium ion channels close and the potassium ion channels open. The membrane is more permeable to potassium so potassium ions diffuse out of the neurone down the potassium ion concentration gradient. This starts to get the membrane back to its resting potential. At the bottom of the curve the potassium ion channels are slow to close so there's a slight 'overshoot' where too many potassium ions diffuse out of the neurone. The potential difference (-70 mV) is more negative than the resting potential (-60 mV). The sodium-potassium pump then returns the membrane to its resting potential (-60 mV).
 b) refractory period

- Q5 The action potential would have the same potential difference values as the graph shown because once the threshold is reached, an action potential will always fire with the same change in voltage, no matter how big the stimulus is. However, there may be another action potential shown on the graph because a bigger stimulus will cause action potentials to fire more frequently.

Page 334 — Fact Recall Questions

- Q1 Sodium-potassium pumps and potassium ion channels.
- Q2 Sodium ions diffuse into the neurone down the sodium ion electrochemical gradient. This makes the inside of the neurone less negative and so decreases the potential difference across the membrane.
- Q3 More sodium ions diffuse into the neurone because more sodium ion channels open.
- Q4 a) The ion channels are recovering and can't be made to open.
 b) It makes action potentials discrete/separate impulses. It means there's a limit to the frequency at which the nerve impulses can be transmitted. It makes action potentials unidirectional.
- Q5 During an action potential, some of the sodium ions that enter the neurone diffuse sideways. This causes sodium ion channels in the next region of the neurone to open and sodium ions diffuse into that part. This causes a wave of depolarisation.
- Q6 A myelinated neurone has a myelin sheath. In the peripheral nervous system the myelin sheath is made of a type of cell called a Schwann cell. Between the Schwann cells are tiny patches of bare membrane called the nodes of Ranvier. Sodium ion channels are concentrated at the nodes of Ranvier.
- Q7 In a myelinated neurone depolarisation/action potentials only happen at the nodes of Ranvier. However in a non-myelinated neurone, depolarisation/action potentials occur as a wave along the whole length of the axon membrane. Conduction along a myelinated neurone is faster than along a non-myelinated neurone.
- Q8 Axon diameter and temperature.

2. Synaptic Transmission

Page 339 — Application Questions

- Q1 The main symptom will be muscle weakness. Calcium ions are unable to enter the synaptic knob. This means the synaptic vesicles won't fuse with the presynaptic membrane so ACh will not be released. Without the release of ACh there will be no action potential triggered in the muscle cell and therefore no response in the muscle.
 If you understand what happens when Ca^{2+} ions do enter the synaptic knob then it should be pretty logical that when they can't enter, the opposite happens.
- Q2 a) They will reduce the sensation of pain. They function as inhibitory neurotransmitters so when they bind to receptors on the postsynaptic membrane it will be hyperpolarised. This means no action potential will be fired and therefore pain signals will not be transmitted.
 b) It will reduce the sensation of pain / it will have the same effect as endorphins. This is because it is very similar in structure to an endorphin molecule so will bind to endorphin receptors and is likely to cause the same effect.

- Q3 Carbachol mimics the action of ACh so the presence of carbachol will activate even more cholinergic receptors. This will make more action potentials fire in the postsynaptic neurone, so more saliva will be produced.

Page 340 — Fact Recall Questions

- Q1 A — synaptic knob, B — vesicle, C — Acetylcholine/ACh, D — presynaptic membrane, E — ACh receptor, F — postsynaptic membrane, G — synaptic cleft.
- Q2 neurone, muscle, gland
- Q3 Receptors are only on postsynaptic membranes. This means the neurotransmitter can't activate an action potential back along the presynaptic neurone.
- Q4 a) The action potential stimulates voltage-gated calcium ion channels in the presynaptic neurone to open, so calcium ions diffuse into the synaptic knob.
b) The influx of calcium ions into the synaptic knob causes the synaptic vesicles to fuse with the presynaptic membrane. The vesicles release ACh into the synaptic cleft. ACh diffuses across the synaptic cleft and binds to specific cholinergic receptors on the postsynaptic membrane. This causes sodium ion channels in the postsynaptic neurone to open. If the threshold is reached, the influx of sodium ions into the postsynaptic membrane causes an action potential on the postsynaptic membrane.
- Q5 So the response doesn't keep happening.
- Q6 An inhibitory neurotransmitter hyperpolarises the postsynaptic membrane, preventing it from firing an action potential.
- Q7 a) Where two or more presynaptic neurones release their neurotransmitters at the same time onto the same postsynaptic neurone, the small amount of neurotransmitter released from each of these neurones can be enough altogether to reach the threshold in the postsynaptic neurone. This makes an action potential more likely.
b) Where two or more nerve impulses arrive in quick succession from the same presynaptic neurone, more neurotransmitter is released into the synaptic cleft. This makes an action potential more likely.
- Q8 A cholinergic synapse between a motor neurone and a muscle cell.
- Q9 E.g. they both release ACh from vesicles in the presynaptic membrane. In both, ACh diffuses across the synaptic cleft and binds to cholinergic receptors on the postsynaptic membrane (which triggers an action potential if the threshold is reached). They both use acetylcholinesterase (AChE) to break down ACh in the synaptic cleft.

3. Muscle Structure

Page 344 — Application Questions

- Q1 The quadriceps because it relaxes when the leg is bent. Remember, the antagonist is the relaxing muscle and the agonist is the contracting muscle. In this case the agonist is the hamstrings — it is contracting, which pulls the lower leg backwards making it bend at the knee.
- Q2 a) B
b) C
c) A and C
d) B
- Q3 Option 1. The A-band has stayed the same length, the I-band is shorter and the H-zone is shorter. Remember, the A-band is the length of the myosin filament and this doesn't get shorter during contraction. During contraction more of the actin filament slides over the myosin filament so the sections with only actin (the I-bands) get shorter and the sections with only myosin (the H-zones) get shorter too.

Page 344 — Fact Recall Questions

- Q1 A pair of muscles that work together to move a bone. One muscle in the pair relaxes as the other contracts.
- Q2 The T-tubules are parts of the sarcolemma that fold inwards across the muscle fibre and stick to the sarcoplasm. They help to spread electrical impulses throughout the sarcoplasm so they reach all parts of the muscle fibre.
- Q3 To provide the ATP that's needed for muscle contraction.
- Q4 An A-band contains myosin filaments and some overlapping actin filaments. Under an electron microscope it appears as a dark band.
- Q5 Myosin and actin filaments slide over one another to make the sarcomeres contract (the myofilaments themselves don't contract).

4. Muscle Contraction

Page 347 — Application Questions

- Q1 a) i) X. The Ca^{2+} concentration is low, suggesting that the muscle is at rest. Muscle fibres are longest when they are relaxed.
ii) Y. There is an influx of Ca^{2+} ions into the sarcoplasm following an action potential, and the Ca^{2+} ions bind to the protein attached to tropomyosin.
iii) Y. The Ca^{2+} ion concentration is high and Ca^{2+} ions activate ATP hydrolase.
- b) The Ca^{2+} ions are moved by active transport from the sarcoplasm back into the sarcoplasmic reticulum, where they're stored.
- c) An action potential from a motor neurone stimulates a muscle cell and depolarises the sarcolemma. Depolarisation spreads down the T-tubules to the sarcoplasmic reticulum, causing the sarcoplasmic reticulum to release stored Ca^{2+} ions into the sarcoplasm.
- Q2 The influx of calcium ions triggers muscle contraction, so more calcium ions in the sarcoplasm would increase the strength of contraction of cardiac/heart muscle, which would help to pump more blood around the body of patients with heart failure.

Page 349 — Application Question

- Q1 a) For the first 26 miles, ATP is likely to be generated via aerobic respiration because the body is being supplied with oxygen. As she sprints the last 385 yards ATP is likely to be generated via anaerobic respiration because the body won't be taking in enough oxygen.
b) Slow twitch. E.g. because they can contract slowly and can work for a long time without getting tired, which makes them good for endurance activities like long-distance running.

Page 349 — Fact Recall Questions

- Q1 E.g. tropomyosin.
Q2 Calcium ions bind to a protein attached to tropomyosin, causing the protein to change shape. This pulls the attached tropomyosin out of the actin-myosin binding site on the actin filament. This exposes the binding site, which allows the myosin head to bind and form an actin-myosin cross bridge.
Q3 ATP is broken down by ATP hydrolase to provide the energy needed to move the myosin head from side to side, which pulls the actin filament along in a rowing action. ATP also provides the energy needed to break the myosin-actin cross bridge, so the myosin head detaches from the actin filament after it's moved.
Q4 a) ATP is made by phosphorylating ADP with a phosphate group taken from PCr.
b) Advantage: e.g. the ATP-PCr system generates ATP very quickly / it can be used during short bursts of vigorous exercise / it's anaerobic/doesn't need oxygen / it's alactic/ doesn't form any lactate.
Disadvantage: e.g. PCr runs out after only a few seconds.
c) Aerobic respiration and anaerobic respiration.
Q5 E.g. they have lots of mitochondria and blood vessels to supply the muscles with oxygen, as they use aerobic respiration. They are rich in myoglobin, a red-coloured protein that stores oxygen.

Exam-style Questions — pages 351-353

- 1.1 Low-intensity exercise uses aerobic respiration, which is how energy is released in slow twitch muscle fibres (**1 mark**).
1.2 Function — e.g. to contract very quickly / to contract powerfully (**1 mark**).
Adaptations — e.g. energy is released quickly through anaerobic respiration using glycogen in fast twitch muscle fibres (**1 mark**). They have stores of PCr so that energy can be generated very quickly when needed (**1 mark**).
1.3 ATP is needed for muscle contraction, so muscle cells need to generate more ATP when they contract (**1 mark**). ATP is generated through respiration, so the rate of respiration in the muscle cell increases (**1 mark**). An increased rate of respiration requires an increased amount of glucose, so more GLUT4 molecules are needed in the cell membrane to transport glucose into the muscle cells (**1 mark**).
1.4 Myosin filaments have globular heads that are hinged (**1 mark**). Each myosin head has a binding site for actin and a binding site for ATP (**1 mark**).
2.1 Action potentials have a refractory period (**1 mark**). During this period the ion channels are recovering and can't be made to open (**1 mark**). This means that no more sodium ions can diffuse into the neurone to trigger another action potential (**1 mark**).
- 2.2 $0.5 \text{ s} = 500 \text{ ms}$
There are 5 action potentials in 20 ms.
 $500 \text{ ms} \div 20 \text{ ms} = 25$. So $5 \times 25 = 125$ action potentials.
(2 marks for correct answer, otherwise 1 mark for correct working)
- 2.3 Axon Y has the biggest diameter as it conducts action potentials faster than axon X (**1 mark**). Action potentials are conducted quicker along axons with bigger diameters because there's less resistance to the flow of ions than in the cytoplasm of a smaller axon (**1 mark**). With less resistance, depolarisation reaches other parts of the neurone cell membrane quicker (**1 mark**).
Still award marks for a correct explanation, even if an incorrect calculation in part 2.2 means that axon X is chosen.
- 3.1 Schwann cell (**1 mark**)
3.2 B — node of Ranvier (**1 mark**)
C — dendrites (**1 mark**)
3.3 Myelin is an electrical insulator (**1 mark**). It allows nervous impulses to travel very fast by saltatory conduction (**1 mark**).
3.4 Conduction of nervous impulses in non-myelinated neurones is slower than in myelinated neurones (**1 mark**). If the myelin is damaged then the nerve impulse may happen much more slowly or not at all, resulting in muscle weakness or paralysis (**1 mark**).
4.1 The biceps and triceps work antagonistically/are an antagonistic pair of muscles (**1 mark**). When the biceps contracts, the triceps relaxes (**1 mark**). This pulls the bone so the arm bends at the elbow (**1 mark**).
4.2 Acetylcholine is released from (the presynaptic membrane of) a motor neurone (**1 mark**) at a neuromuscular junction (**1 mark**). It then diffuses across the synaptic cleft (**1 mark**) and binds to nicotinic cholinergic receptors (**1 mark**) on the motor end plate/postsynaptic membrane, causing the postsynaptic/muscle cell to depolarise (**1 mark**).
4.3 A — H-zone (**1 mark**), B — I-band (**1 mark**), C — A-band (**1 mark**)
4.4 A/the H-zone and B/the I-band will appear longer (**1 mark**) and C/the A-band will stay the same length (**1 mark**).
4.5 ATP is made by phosphorylating ADP (**1 mark**) with a phosphate group taken from phosphocreatine/PCr (**1 mark**). Remember, the ATP-phosphocreatine (PCr) system is used during short bursts of vigorous exercise.
4.6 E.g. ATP is generated very quickly (**1 mark**). / No oxygen is needed / the process is anaerobic (**1 mark**). / The process is alactic / no lactate is formed (**1 mark**).
5.1 Time 1 shows repolarisation (**1 mark**) because the sodium ion channels are closed and the potassium ion channels are open (**1 mark**). The membrane is more permeable to potassium so potassium ions diffuse out of the neurone down their concentration gradient (**1 mark**). Time 2 shows hyperpolarisation/the refractory period (**1 mark**) because both the sodium and potassium ion channels are closed (**1 mark**). There is no movement of sodium or potassium through their ion channels (by facilitated diffusion) (**1 mark**). If a question tells you to 'use evidence' from a source (like a diagram, graph, table, etc.) this means you need to include figures or descriptions using the source. So in this case, you need to say which ion channels are open and closed in Figure 4.
5.2 Sodium-potassium pumps use active transport to move three sodium ions out of the cell (**1 mark**) for every two potassium ions moved in (**1 mark**).
5.3 The potassium ion channel is slow to close so too many potassium ions diffuse out of the neurone (**1 mark**). The potential difference is more negative than the neurone cell membrane's resting potential, so the pump returns the membrane to its resting potential (**1 mark**).

- 5.4 It would be faster at 30 °C than at 20 °C (**1 mark**) because the ions would diffuse faster across the membrane so depolarisation would reach other parts of the neurone cell membrane more quickly (**1 mark**).
- 5.5 temporal summation (**1 mark**)
- 5.6 More acetylcholine/ACh will be released into the synaptic cleft, which means more acetylcholine/ACh will bind to receptors on the postsynaptic membrane (**1 mark**). This causes more sodium ion channels to open and a greater influx of sodium ions (**1 mark**), which makes the postsynaptic neurone more likely to reach threshold and fire an action potential (**1 mark**).
The question asks specifically about a cholinergic synapse so you should be specific in your answer and write acetylcholine (or ACh) rather than just putting 'neurotransmitter'.
- 5.7 When a stimulus excites the neurone, sodium ions won't be able to diffuse into the neurone through sodium ion channels (**1 mark**). This means that the threshold level won't be reached (**1 mark**) so there will be no action potentials/no nervous impulses (**1 mark**).

Topic 6 — C: Homeostasis

1. Homeostasis Basics

Page 358 — Application Questions

Q1 a) $\text{pH} = -\log_{10} (5.50 \times 10^{-8}) = 7.26$

Yes, the patient could be suffering from metabolic acidosis (as the patient's blood pH is below 7.35).

The value given (5.50) is written to three significant figures, so your answer should also be given to three significant figures.

b) Enzymes in the blood work best within the normal blood pH range. If the blood pH becomes too high or too low, the ionic bonds and hydrogen bonds supporting the shape of the enzymes' active sites will be broken and the enzymes will become denatured. This means that they won't be able to catalyse metabolic reactions.

Q2 A is an example of negative feedback because increasing respiration rate will increase the rate at which carbon dioxide is removed from the body. This will increase the pH of the blood back to the normal level. B is an example of positive feedback because more oestrogen being released will increase the levels of LH further and amplify the change.

Page 358 — Fact Recall Questions

- Q1 The maintenance of a stable internal environment.
- Q2 So that metabolic reactions can occur at an optimum rate. Low temperatures make metabolic reactions slower, but if the temperature gets too high the reaction essentially stops.
- Q3 If blood glucose concentration is too high the water potential of blood is reduced to a point where water molecules diffuse out of cells into the blood by osmosis. This can cause the cells to shrivel up and die. If blood glucose concentration is too low, cells are unable to carry out normal activities because there isn't enough glucose for respiration to provide energy.
- Q4 A positive feedback mechanism amplifies a change from the normal level, whereas a negative feedback mechanism restores the level to normal.

2. Control of Blood Glucose Concentration

Page 362 — Application Questions

- Q1 Carbohydrates in the pasta are broken down into glucose, so their blood glucose concentration will increase. When the pancreas detects the blood glucose concentration is too high, the β cells will secrete insulin and the α cells will stop secreting glucagon. Insulin will then bind to receptors on liver and muscle cells (the effectors). These cells will respond by taking up more glucose, activating glycogenesis and by respiring more glucose. Blood glucose concentration will then return to normal.
- Q2 Glycogenolysis and gluconeogenesis both increase blood glucose concentration. If these processes don't work properly then when blood glucose concentration falls (i.e. if the person doesn't eat regularly) the body will be unable to raise the blood glucose concentration back to normal, so the person will suffer from hypoglycaemia.

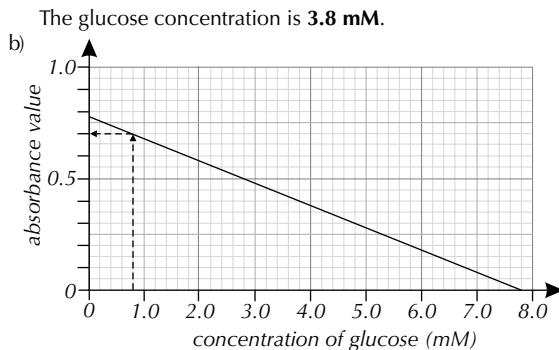
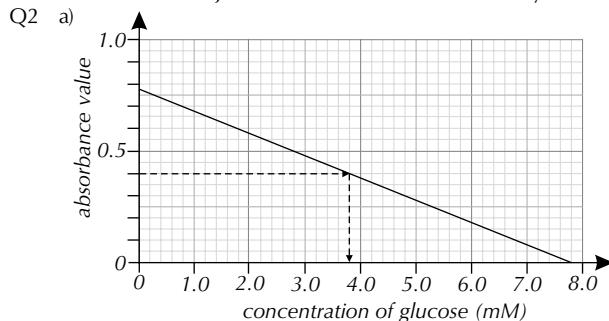
Page 362 — Fact Recall Questions

- Q1 Insulin is secreted from the β cells and glucagon from the α cells of the islets of Langerhans in the pancreas.
- Q2 It increases the permeability of muscle cell membranes to glucose by making glucose transporters available, activates enzymes in liver and muscle cells that convert glucose into glycogen/activates glycogenesis and increases the rate of respiration of glucose in those cells.
- Q3 glycogenesis
- Q4 Gluconeogenesis — glycerol and amino acids are converted to glucose. Glycogenolysis — glycogen is converted to glucose.
- Q5 The pancreas detects blood glucose is too low. α cells secrete glucagon and β cells stop secreting insulin. Glucagon binds to receptors on liver cells. The liver cells respond to increase the blood glucose concentration (e.g. glycogenolysis is activated), so blood glucose concentration returns to normal.
- Q6 When insulin binds to receptors on the muscle cell membrane, it triggers the movement of GLUT4/glucose transport proteins (channel proteins) from vesicles in the cytoplasm of the cell to the cell membrane. Glucose can then be transported into the cell through these channel proteins by facilitated diffusion.
- Q7 Adrenaline binds to its specific receptors on the cell surface membrane (of liver cells), which causes an enzyme called adenylate cyclase to be activated inside the cell. Activated adenylate cyclase converts ATP into a second messenger called cAMP. cAMP activates an enzyme called protein kinase A, which activates a cascade (chain of reactions) that breaks down glycogen into glucose.

3. Diabetes and Blood Glucose Concentration

Pages 366-367 — Application Questions

- Q1 a) Day 1 because blood glucose concentration increased more after lunch on day 1 (by 4.5 mM) than it did after lunch on day 2 (by 3.8 mM).
b) E.g. the same dose of insulin was injected on both days. / Insulin was injected at the same time on both days.



The highest 'normal' glucose concentration (0.8 mM) would be expected to have an absorbance value of 0.7, according to the graph. To find the absorbance of the lowest 'normal' glucose concentration (0 mM), just look at where the calibration curve meets the y-axis.

Page 367 — Fact Recall Questions

- Q1 The immune system attacking the β cells in the islets of Langerhans so they can't produce any insulin.
Q2 Any two from: e.g. obesity / family history / lack of exercise / more advanced age / poor diet.
Q3 By eating a healthy, balanced diet, losing weight if necessary, exercising regularly or taking glucose-lowering medication if these don't work. Insulin injections may be needed eventually.
Q4 E.g. reduce the advertising of junk food, improve the nutritional value of their products, and use clearer labelling on products.
Q5 E.g. do the Benedict's test on each solution using quantitative Benedict's reagent, then measure the absorbance of each solution using a colorimeter (with a red filter). Next plot the absorbance of each solution against the glucose concentration and draw a calibration curve. Then do the Benedict's test on the urine sample using quantitative Benedict's reagent (under the same conditions as used for the serial dilutions, e.g. the same volume of solution and Benedict's reagent, the same temperature, the same amount of time) and measure its absorbance. Finally, use the calibration curve to find the concentration of glucose in the urine sample.

4. The Kidneys

Page 370 — Application Questions

- Q1 A — Bowman's capsule
B — loop of Henle
C — distal convoluted tubule/DCT
D — ureter
- Q2 a) X — basement membrane
Y — epithelium of Bowman's capsule / podocyte
b) E.g. the structure of the barrier normally prevents larger molecules such as proteins from entering the tubules. If its structure is affected, large molecules such as proteins may be able to pass into the tubules and eventually end up in the urine, producing proteinuria.

Page 370 — Fact Recall Questions

- Q1 a) afferent arteriole
b) Bowman's capsule
c) Because vessel A/the afferent arteriole is larger in diameter than vessel B/the efferent arteriole, the blood in the glomerulus is under high pressure. The high pressure forces liquid and small molecules in the blood out of the capillary and into the Bowman's capsule (ultrafiltration). If you're struggling to remember the difference between the afferent and efferent arterioles, think afferent comes first, because it's first alphabetically.
- Q2 e.g. water and glucose

5. Controlling Blood Water Potential

Page 373 — Application Questions

- Q1 a) The runner is dehydrated because he has sweated a lot and not replaced any of the fluids he has lost. This has caused his blood water content to drop.
b) The low water content of the runner's blood is detected by osmoreceptors in his hypothalamus.
c) ADH molecules bind to receptors on the plasma membranes of cells of the runner's distal convoluted tubule/DCT and collecting duct. When this happens, protein channels called aquaporins are inserted into the plasma membrane. These channels allow water to pass through via osmosis, so make the walls of the DCT and collecting duct more permeable to water. This allows water to be reabsorbed from these tubules into the medulla and into the blood by osmosis, therefore conserving water in the runner's body.
d) The presence of sodium (Na^+) ions in the sports drink increases the concentration of Na^+ in the runner's glomerular filtrate. These ions are used to lower the water potential of the medulla in the loop of Henle in order to create a water potential gradient to drive the reabsorption of water back into the blood by osmosis.

Make sure you understand water potential. If you don't, it makes understanding the regulation of water content by the kidneys pretty tricky. Remember, high water potential means a high concentration of water molecules and low water potential means a low concentration of water molecules. Water moves from a region of higher water potential to a region of lower water potential — from where there are more water molecules to where there are fewer.

- Q2 a) Normally if a person has consumed too much fluid, the osmoreceptors in the hypothalamus detect that the water content of the blood, and so its water potential, has risen. This causes the posterior pituitary gland to release less ADH into the blood. Less ADH means that the DCT and collecting duct are less permeable, so less water is reabsorbed into the blood by osmosis. This causes a large amount of dilute urine to be produced and so more water is lost.
- b) If the body can't suppress ADH production, the DCT and collecting duct will continue to be made permeable, so water is reabsorbed into the blood by osmosis. This means that the excess water is not excreted and therefore accumulates, potentially affecting the balance of fluid in cells.
- Q3 a) E.g. a longer loop of Henle means that more water can be reabsorbed from the glomerular filtrate, so the fennec fox can conserve as much water as possible.
- b) E.g. frogs and toads live in wet environments, so they don't need to conserve water by reabsorbing it from the glomerular filtrate.

Page 373 — Fact Recall Questions

- Q1 The control of the water potential of the blood.
 Q2 the medulla
 Q3 the ascending limb
 Q4 the hypothalamus

Exam-style Questions — pages 375-377

- 1.1 At point A low concentrations of calcium in the blood are detected, which stimulates the secretion of PTH (**1 mark**). At point B effectors are responding by increasing the concentration of calcium in the blood (**1 mark**). At point C high concentrations of calcium in the blood are detected, which stimulates the secretion of calcitonin (**1 mark**). At point D effectors are responding by decreasing the concentration of calcium in the blood (**1 mark**).
- 1.2 Having more than one negative feedback mechanism means there is more control over changes in the blood calcium concentration (**1 mark**). It means that the blood calcium concentration can be actively increased or decreased to return it to normal rather than just changing it in one direction (**1 mark**).
- 1.3 The concentration of calcium in the blood may fall very low (**1 mark**). This is because less PTH will be released to bring the levels back up to normal (**1 mark**).
- 2.1 Useful substances are reabsorbed back into the blood from the tubules / selective reabsorption takes place (**1 mark**).
- 2.2 Microvilli (**1 mark**). The epithelium of the wall of the PCT has microvilli to provide a large surface area for the reabsorption of useful materials from the glomerular filtrate into the blood (**1 mark**).
- 2.3 glomerulus (**1 mark**)
 2.4 E and F (**1 mark**)
 2.5 Loop of Henle (**1 mark**). It controls the movement of sodium ions so that water can be reabsorbed by the blood. (**1 mark**).
- 3.1 α cells in the pancreas are secreting glucagon into the blood (**1 mark**). β cells stop secreting insulin (**1 mark**).

- 3.2 The Type II diabetic doesn't produce as much insulin as the non-diabetic. /The body's cells don't respond properly to the insulin that's produced (**1 mark**). Insulin lowers blood glucose concentration when it's too high, so if there's not enough insulin / the body can't respond to insulin properly, this process will be much slower (**1 mark**).
- 3.3 More quickly, because more glucose is respirated during exercise to provide energy (**1 mark**).
- 3.4 A Type I diabetic wouldn't produce any insulin (**1 mark**). This means that blood glucose concentration would remain high for much longer than for this Type II diabetic (**1 mark**).
- 3.5 22.5 minutes (**1 mark**). This is because this is the time when the blood glucose concentration is at its upper limit / $110 \text{ mg}/100 \text{ cm}^3$ (**1 mark**). You're told the normal range for blood glucose concentration in the introduction to the question — make sure you always read questions thoroughly in the exam.
- 3.6 Insulin is a hormone, so it takes time to travel in the blood to receptor cells (**1 mark**).
- 3.7 When insulin binds to receptors on a target cell, it triggers the movement of channel proteins/the glucose transporter GLUT4 from vesicles in the cytoplasm to the cell membrane (**1 mark**). Glucose can then be transported into the cell by facilitated diffusion (**1 mark**).
- 3.8 The hormones glucagon and adrenaline bind to specific receptors on liver cells (**1 mark**). This activates the enzyme adenylate cyclase (**1 mark**), which converts ATP into the second messenger, cyclic AMP (cAMP) (**1 mark**). Cyclic AMP then activates an enzyme called protein kinase A (**1 mark**), which activates a cascade that initiates glycogenolysis in the cell (**1 mark**).

| Substance | TF/P ratio of 1.0 |
|-------------------------------|-------------------|
| urea | ✓ |
| serum albumin (protein) | ✗ |
| sodium ions (Na^+) | ✓ |
| glucose | ✓ |
| red blood cells | ✗ |

(**2 marks for all three correct, 1 mark for 2 correct.**)

Don't let the numbers throw you in this question. All you're really being asked is which substances can cross the filtration barrier and which can't.

- 4.2 Normally proteins like serum albumin can't pass through the filtration barrier into the tubular fluid because they are too large, so it stays in the blood (**1 mark**).
- 4.3 The reabsorption of Na^+ from the kidney tubule back into the capillaries lowers the water potential of the medulla (**1 mark**). This drives the reabsorption of water from the kidney tubule via osmosis (**1 mark**). If the amount of sodium reabsorbed is decreased then the amount of water reabsorbed will also decrease (**1 mark**). This means more water will be removed from the body in the urine, lowering the water content and therefore the volume of the blood (**1 mark**).

Topic 7

Topic 7 — A: Genetics

1. Genetic Terms

Page 379 — Application Questions

Q1 A = tufted tail, B = tufted tail, C = non-tufted tail

Q2 a) yellow

b) YY

c) yy

You need to understand all the terms on pages 378 and 379 really well so that when you come across exam questions using any of the words, you'll understand what's being described and what you're being asked.

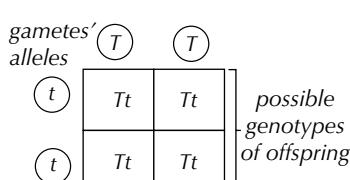
2. Genetic Diagrams — Simple Monohybrid Crosses

Page 382 — Application Questions

Q1 The only possible genotype of offspring is heterozygous, e.g. Tt. Worked example:

T — tall dominant allele

t — dwarf recessive allele

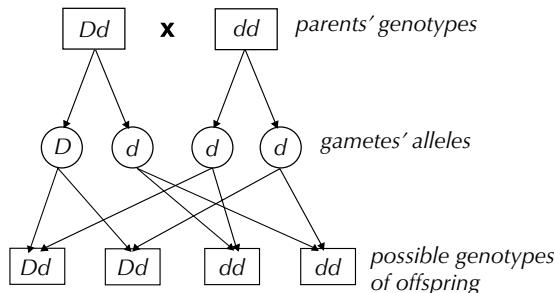


The question asked you to show your working. So even though you know that a monohybrid cross with two homozygous parents always produces all heterozygous offspring, you must draw a genetic diagram of some kind to show how you would work that out.

Q2 $\frac{1}{2} / 0.5 / 50\%$. Worked example:

D — polydactyl dominant allele

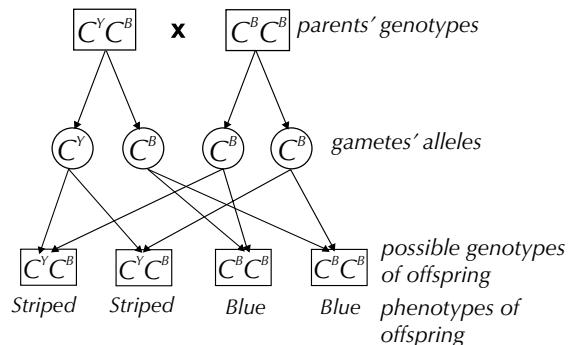
d — normal recessive allele



Q3 1 : 0 : 1 ratio of blue : yellow : striped organisms / 1 : 1 ratio of blue : striped organisms. Worked example:

C^Y — yellow allele

C^B — blue allele

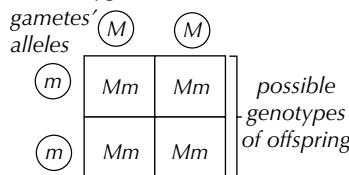


3. Genetic Diagrams — Multiple Allele and Dihybrid Crosses

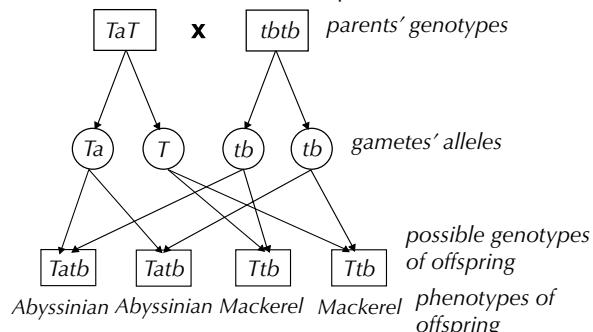
Page 385 — Application Questions

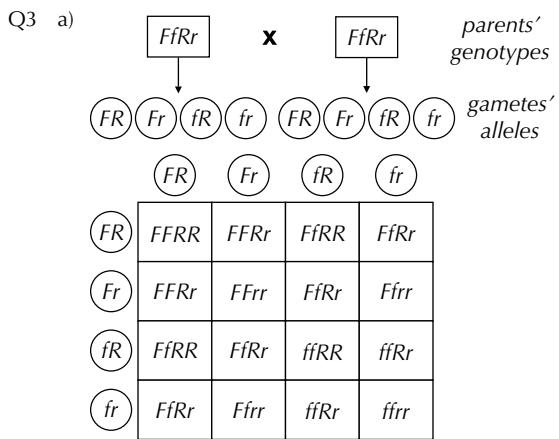
Q1 a) Melanic (M) is dominant to both of the other alleles, insularia is dominant to typical only and typical is recessive to all. / The dominance of the alleles is $M > M' > m$.

b) All heterozygous melanic (Mm). Worked example:



Q2 The possible striping patterns are Abyssinian (50%) and Mackerel (50%). Worked example:



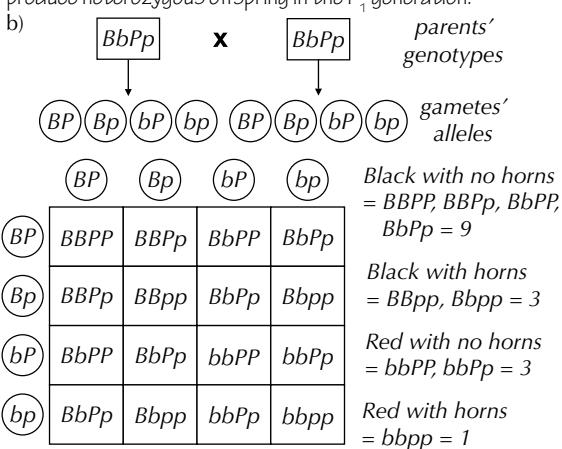


b) 9 : 1 ratio of round, red tomatoes to pear-shaped, yellow tomatoes.

Round, red tomatoes = FFRR, FFRr, FfRR, FfRr

Pear-shaped, yellow tomatoes = ffr

Q4 a) The offspring would all be heterozygous (BbPp).
The red cow with horns must have a homozygous recessive phenotype (bbpp). Dihybrid crosses between homozygous dominant and homozygous recessive parents will always produce heterozygous offspring in the F₁ generation.



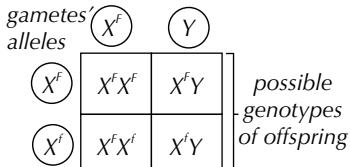
Phenotypic ratio: 9 : 3 : 3 : 1

4. Linkage

Page 389 — Application Questions

Q1 X^FX^F (affected female), X^FY (affected male), X^FX^f (affected female), X^fY (unaffected male). Worked example:

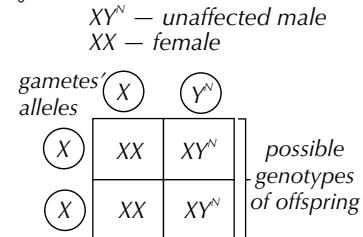
X^FY — affected male
X^FX^f — affected heterozygous female



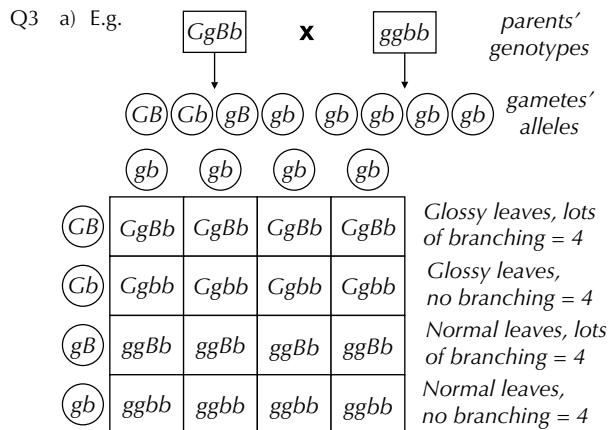
This question doesn't ask you to show your working, but it's best to always do so. Then if you write an answer down wrong for any reason, you could still pick up marks in your exam for your working.

Q2 Y-linked characteristics can only be passed on down the male (XY) line. So for a child to have a Y-linked disorder, its father must also have the disorder. So if a child has hairy ears but its dad doesn't, the dad might question if he was the father.

This is fairly tricky, but drawing a quick diagram would help you out:



An unaffected male can't have a child with the disorder.



Expected phenotypic ratio = 1 : 1 : 1 : 1

b) Observed phenotypic ratio = 1.7 : 1.1 : 1 : 1.8

c) The GB alleles and the gb alleles in the GgBb parent may have been linked. This would mean that the GgBb parent produced mostly GB and gb gametes and make the GgBb and ggbb genotypes more common in the offspring. As a result, a higher proportion of the offspring would have their parents' phenotypes, instead of the even split of phenotypes predicted.

Page 389 — Fact Recall Questions

Q1 1/2 / 0.5 / 50%

Q2 If a characteristic is sex-linked it means that the allele that codes for it is located on a sex chromosome (X or Y).

Q3 Males are more likely than females to have X-linked disorders because males only have one X chromosome. Because they only have one copy of any alleles on the X chromosome, they express the characteristic of those alleles even if they're recessive, whereas women would need to inherit two copies to express the same characteristics.

Q4 An autosome is any chromosome that isn't a sex chromosome.

Q5 Genes on the same autosome are said to be linked because being on the same autosome usually means they'll stay together during the independent segregation of chromosomes in meiosis I. This means that their alleles will be passed on to the offspring together (unless crossing over splits them up first).

5. Epistasis

Page 392 — Application Questions

Q1 a) i) EEBB, EeBB, EEBb, EeBb

For the dog to be black it must be able to express the dark pigment, so it must have at least one dominant E allele. Also, it must have at least one copy of the dominant B allele for the black pigment to be shown in the phenotype.

ii) eebb, EEbb

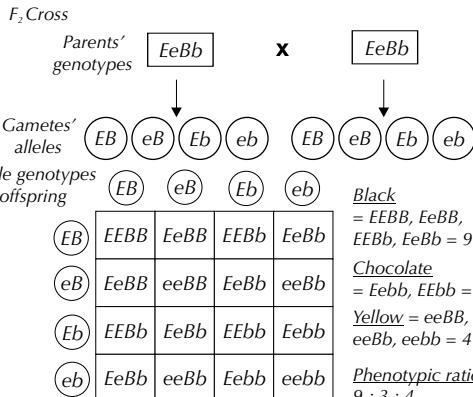
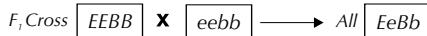
For the dog to be chocolate it must be able to express the dark pigment, so it must have at least one dominant E allele. Also, it must have two copies of the recessive b allele for the chocolate pigment to be shown in the phenotype.

iii) eeBB, eeBb, eebb

For the dog to be yellow it must have two copies of the recessive e allele, so that it can't express the dark pigment. Gene 1 is epistatic over gene 2, so it doesn't matter what B or b alleles the dog has — it will still be yellow.

b) A cross between EEBB and eebb parents will give a 9 : 3 : 4 phenotypic ratio in the F₂ generation of black : chocolate : yellow. This is because gene 1 has a recessive epistatic allele (e) and two copies of the recessive epistatic allele (ee) will mask the expression of gene 2. Here's the cross to prove it:

B = black pigment, b = chocolate pigment, E = can express dark pigment, e = can't express dark pigment



In the exam, you wouldn't need to draw out the genetic cross unless the question specifically asked you to. We've just included it here to help you out.

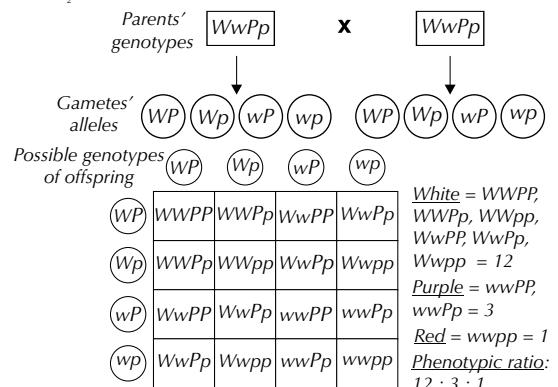
Q2 a) dominant epistasis

b) A cross between WWPP and wwpp produces a 48 : 12 : 4 or 12 : 3 : 1 phenotypic ratio in the F₂ generation of white : purple : red. This is because gene 1 has a dominant epistatic allele (W) and one or more copies of the dominant epistatic allele (Ww or WW) will mask the expression of gene 2.

c) W = white pigment, w = red pigment, P = purple pigment, p = no purple pigment

F₁ Cross $\boxed{WWPP} \times \boxed{wwpp} \longrightarrow \text{All } \boxed{WwPp}$

F₂ Cross



6. The Chi-Squared Test

Pages 396 — Application Questions

Q1 a) Yes, the difference would be significant because the chi-squared value is greater than the critical value ($6.20 > 5.99$).

If the difference is significant it means the difference is unlikely to be due to chance and that the null hypothesis is rejected.

b) No, the difference would not be significant because the chi-squared value is smaller than the critical value ($4.85 < 5.99$).

If the difference is not significant it means the difference is likely to be due to chance — we're unable to reject the null hypothesis.

Q2 a)

| Phenotype | Ratio | Expected result (E) | Observed result (O) | O - E | $(O - E)^2 / E$ |
|------------------|-------|---------------------|---------------------|-------|-----------------|
| Round, green | 9 | 72 | 74 | 2 | 0.06 |
| Round, yellow | 3 | 24 | 21 | -3 | 0.38 |
| Wrinkled, green | 3 | 24 | 26 | 2 | 0.17 |
| Wrinkled, yellow | 1 | 8 | 7 | -1 | 0.13 |
| $\chi^2 = 0.74$ | | | | | |

b) There are 4 phenotypes which means there are $4 - 1 = 3$ degrees of freedom. From the table, the critical value for a test with 3 degrees of freedom and a 0.05 probability level is 7.82. The chi-squared value is smaller than the critical value ($0.74 < 7.82$) so the difference between the observed and expected results is not significant. This means we're unable to reject the null hypothesis.

- Q3 a) There is no significant difference between the observed and the expected results.
 b) This is unlikely to be an example of codominance.
 There are 3 phenotypes which means there are $3 - 1 = 2$ degrees of freedom. From the table, the critical value for a test with 2 degrees of freedom and a 0.05 probability level is 5.99. The chi-squared value is greater than the critical value ($8.6 > 5.99$) so the difference between the observed and expected results is significant. This means that the null hypothesis can be rejected.

Exam-style Questions — page 398-399

- 1.1 Number of agouti offspring = $(256 \div 4) \times 3 = 192$ (1 mark)

A normal case of monohybrid inheritance would give a phenotypic ratio of 3 : 1 of agouti : solid coloured. So three quarters of the offspring would have agouti coat colour.

- 1.2 ppAA, ppAa, ppaa (1 mark)

- 1.3 A cross between PPAA and ppaa parents will give a 9 : 3 : 4 phenotypic ratio in the F_2 generation of agouti : solid coloured : albino (1 mark). This is because the P gene has a recessive epistatic allele (p) and two copies of the recessive epistatic allele (pp) will mask the expression of the pigmentation gene (1 mark). A dihybrid cross will only give a phenotypic ratio of 9 : 3 : 3 : 1 in the F_2 generation if the two genes do not interact and are not linked (1 mark).

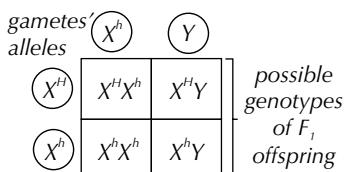
2.1

| Genotype | Growth on substance 1 |
|----------|-----------------------|
| AaBb | ✓ |
| aaBb | ✗ |
| AAbb | ✗ |
| AABb | ✓ |

(1 mark for all 3 correct)

- 2.2 They must have the genotype aaBb (or aaBB) (1 mark). They can't produce enzyme A but can produce enzyme B (1 mark), so if they're given substance 2 they can convert it to substance 3 (1 mark).

- 3.1 X^hY — haemophiliac male
 X^hX^h — female carrier



Possible phenotypes of F_1 offspring: carrier female (X^hX^h), normal male (X^hY), haemophiliac female (X^hX^h) and haemophiliac male (X^hY).

(1 mark for correct gametes, 1 mark for correct F_1 genotypes, 1 mark for F_1 phenotypes matched to correct F_1 genotypes.)

- 3.2 The difference between the observed and expected results is not significant because the critical value for this test at $P = 0.05$ is 7.82 (1 mark) and the chi-squared value (1.04) is less than this (1 mark).

Topic 7 — B: Populations and Evolution

1. The Hardy-Weinberg Principle

Page 404 — Application Questions

- Q1 a) From the bar chart, $p = 0.10$

$$p + q = 1$$

$$q = 1 - p$$

$$q = 1 - 0.10 = 0.90$$

You're given one allele frequency in the bar chart and are asked to find the other, so it's the simple equation.

$$b) p = 0.1, q = 0.9, \text{ so } 2pq = 2 \times 0.1 \times 0.9 = 0.18$$

c) No, it does not apply. The frequency of the allele changes between the generations, and the Hardy-Weinberg principle is only true in cases where the allele frequency stays the same.

- Q2 Say p = frequency of H^S , q = frequency of H^N .

$$\text{frequency of genotype } H^S H^S = p^2 = 1 \div 500 = 0.002 \\ \text{so } p = \sqrt{0.002} = 0.044\dots$$

$$q = 1 - p$$

$$q = 1 - 0.044\dots = 0.955\dots$$

Heterozygotes have sickle cell trait, so the frequency of sickle cell trait is:

$$2pq = 2 \times 0.044\dots \times 0.955\dots = 0.09 \text{ (2 d.p.)}$$

H^S and H^N are codominant, so it doesn't matter which letter (p or q) you pick to represent the frequency of which allele (H^S or H^N). Just write down which letter you've assigned to which allele so you don't get confused.

- Q3 $q = 0.16$ and $p + q = 1$, so $p = 1 - q$

$$p = 1 - 0.16 = 0.84$$

homozygous dominant genotype frequency = p^2

$$p^2 = 0.84^2 = 0.71 \text{ (2 d.p.)}$$

- Q4 recessive wrinkled allele = $q^2 = 31\% \div 100 = 0.31$

$$q = \sqrt{0.31} = 0.556\dots$$

$$p + q = 1, \text{ so } p = 1 - q$$

$$p = 1 - 0.556\dots = 0.443\dots$$

Heterozygous genotype = $2pq$

$$2pq = 2 \times 0.443\dots \times 0.556\dots = 0.49 \text{ (2 d.p.)}$$

$0.49 \times 100 = 49$, so 49% of the population have a heterozygous genotype.

Page 404 — Fact Recall Questions

- Q1 A group of organisms of the same species living in a particular area.

- Q2 The complete range of alleles present in a population.

- Q3 How often an allele occurs in a population.

- Q4 The Hardy-Weinberg principle is a mathematical model that predicts that the frequencies of alleles in a population won't change from one generation to the next as long as the population is large, there's no immigration, emigration, mutations or natural selection, and mating is totally random.

- Q5 $p + q = 1$ and $p^2 + 2pq + q^2 = 1$, where p = the frequency of the dominant allele, q = the frequency of the recessive allele, p^2 = the frequency of the homozygous dominant genotype, q^2 = the frequency of the homozygous recessive genotype and $2pq$ = the frequency of the heterozygous genotype.

2. Variation and Selection

Page 407 — Application Question

- Q1 a) $3.5 \text{ cm} - 1 \text{ cm} = 2.5 \text{ cm}$
- b) Directional selection. In 1850 the average fur length was about 3.5 cm. In 1950, the average had moved to about 2.2 cm. The average has moved towards the 'extreme' shorter end, which shows directional selection.
- c) Caribou with shorter fur length would have been better adapted to the warmer climate further south and so had a selective advantage. These caribou would have been more likely to survive, reproduce and pass on their alleles for shorter fur. So the average fur length has reduced and moved towards the 'extreme' shorter end.

Page 407 — Fact Recall Questions

- Q1 Genetic variation can be caused by mutation (which leads to the production of new alleles), during meiosis, and through random fertilisation of gametes during sexual reproduction. Remember, the main source of genetic variation is mutation.
- Q2 Competition, predation and disease are selection pressures which create a struggle for survival. Members of a species show variation in their alleles, which makes some individuals better adapted to these selection pressures than others. These individuals are more likely to survive, reproduce, and pass on their beneficial alleles to the next generation compared to individuals that are not as well-adapted. In this way allele frequencies in the gene pool change as the frequency of the beneficial alleles increases over time.
- Q3 Stabilising selection is when individuals with alleles for a characteristic towards the middle of the range are more likely to survive, reproduce and pass on their alleles, whereas disruptive selection is when individuals with alleles for characteristics at either of the extreme ends of a range are more likely to survive and reproduce than individuals with alleles for characteristics in the middle of the range. This means that stabilising selection causes the range of values of a phenotype to narrow around the existing mean, whereas disruptive selection causes phenotypes at the extreme ends of a range to increase in frequency, and phenotypes in the middle of the range to decrease in frequency.

3. Speciation and Genetic Drift

Pages 411-412 — Application Questions

- Q1 Different populations were geographically isolated on the different islands. The different food sources caused different selection pressures, so on each island finches with different alleles for beak size and shape were more likely to survive, reproduce and pass on their alleles. Over time this caused changes in allele frequencies and gene pools, which eventually resulted in reproductive isolation and allopatric speciation.
- Q2 Genetic drift could have a large effect in the Mauritian pink pigeon population because it is very small. This could lead to a loss of genetic diversity, which could make the species less able to adapt to changes in the environment. This may put the species at an even greater risk of extinction.
- Q3 a) The frequency of pink eyes in the island population falls across the study period (eventually reaching zero), whereas the frequency of pink eyes in the mainland population remains roughly constant. The change in the pink eye frequency on the island is likely to be due to genetic drift. This can occur more easily on the island than on the mainland as the population on the island is smaller.
- You're told in the question that the colour of the rodents' eyes are not thought to affect the chances of survival or breeding, so this indicates that natural selection is not likely to be responsible for any changes in the frequency of eye-colour phenotypes.
- b) Male rodents on the island are likely to have become larger over time via natural selection. This is because a larger size means the rodents are more likely win territorial fights, so they're more likely to survive, reproduce and pass on their beneficial alleles for a larger size to the next generation. This may not have happened in the mainland population because a larger size also makes the rodents more visible in the undergrowth, which makes them more susceptible to predation. So on the mainland (where there are mammalian predators), having a larger size may not make an individual more likely to survive, so this trait may not have become more common via natural selection.
- c) E.g. they could investigate whether rodents from the island can breed successfully with rodents from the mainland and produce fertile offspring.

Page 412 — Fact Recall Questions

- Q1 Speciation is the development of a new species from an existing species.
- Q2 Allopatric speciation — this occurs when populations of the same species become geographically isolated and eventually become reproductively isolated. Sympatric speciation — this occurs when a population becomes reproductively isolated even though there is no geographical isolation.
- Q3 E.g. behavioural changes to courtship rituals could make a group of individuals unattractive to the main population, preventing them from mating together, even if they could breed successfully.
- Q4 Initially, there was one population of organisms. This population was divided, and the two populations evolved into separate species. This process repeated itself many times over a long period of time, leading to the diversity of life on Earth we see today.

Exam-style Questions — page 414

- 1.1 A population is a group of organisms of the same species living in a particular area at a particular time, that have the potential to breed with each other (**1 mark**).
- 1.2 The variation could be caused by genetic factors/individuals having different alleles (**1 mark**). Genetic variation is most likely to be caused by mutation, in which changes in an individual's DNA base sequence lead to the production of new alleles (**1 mark**). Genetic variation within a species may also be caused by meiosis (**1 mark**) and by the random fertilisation of gametes during sexual reproduction (**1 mark**).
- 1.3 This means that changes in allele frequency have caused changes in the phenotypes of the two species of fish (**1 mark**), which means they cannot interbreed to produce fertile offspring (**1 mark**). These two species may be reproductively isolated because of e.g. differences in their behaviour / differences in the time of year when they breed / differences in their genes that make offspring infertile/ inviable (**1 mark**).
- 1.4 Sympatric speciation (**1 mark**) because the new species/A. *zaliosus* arose whilst still living in the same geographic area as the original species/A. *citrinellus* (**1 mark**).
- 1.5 In disruptive selection two phenotypes are favoured by the environment, so individuals that show either of these two phenotypes can survive and reproduce (**1 mark**). In Lake Apoyo, it could have been that individuals with phenotypes that made them specialised to live in the open water column or on the lake bed were most likely to survive and reproduce (**1 mark**), so the alleles for these two phenotypes increased in frequency (**1 mark**). This could have resulted in two separate populations of A. *citrinellus* which were likely to only breed with other fish from their own population (**1 mark**). Eventually, changes in allele frequency between the two populations may have led to the fish becoming reproductively isolated and a new species/A. *zaliosus* developing (**1 mark**).
- 2.1 $q^2 = 5 \div 1000 = 0.005$
 $q = \sqrt{0.005} = 0.070\dots$
 $p = 1 - 0.070\dots = 0.929\dots$
 $2pq = 2 \times 0.929\dots \times 0.070\dots = 0.1314\dots$
 $0.1314\dots \times 100 = 13.14\%$ (2 d.p.)
(2 marks for the correct answer, otherwise 1 mark for identifying $2pq$ as the frequency of heterozygotes in the population.)
- 2.2 Genetic drift (**1 mark**). The syndrome does not increase a person's chance of surviving, so the allele must have become more common in the population by chance (**1 mark**).

Topic 7 — C: Populations in Ecosystems

1. Ecosystems

Pages 416-417 — Application Questions

- Q1 a) abiotic conditions
The kangaroo rat is adapted to a lack of water in its habitat — which is a non-living feature, and so an abiotic condition.
- b) By producing concentrated urine the kangaroo rat is able to conserve water. This helps it to survive in deserts where there isn't much water available.
- Q2 a) The length of their probosci.
b) The new bee species would compete with the current species with a long proboscis. One species would compete more successfully until only it is left / until it has out-competed the other species.
- Q3 a) Species A has a small beak length, with most individuals having a beak length of around 5 mm. Species B has a longer beak, with most individuals having a beak length of around 15 mm (a difference of 10 mm).
b) The two bird species occupy different niches. Their beaks are different sizes, which could mean that they eat different sized seeds, so they wouldn't be in competition with each other.

Page 417 — Fact Recall Questions

- Q1 All the organisms living in a community and all the non-living/abiotic conditions found there.
- Q2 The place where an organism lives within an ecosystem.
- Q3 The role of a species within its habitat.
- Q4 a) E.g. the organisms a species eats, the organisms a species is eaten by.
b) E.g. the temperature range an organism can live in, the time of day when an organism is active.
- Q5 A feature that increases an organism's chance of survival and reproduction.
- Q6 a) Organisms with better adaptations are more likely to survive, reproduce and pass on the advantageous alleles for their adaptations, so the adaptations become more common in the population.
b) natural selection

2. Variation in Population Size

Page 422 — Application Questions

- Q1 The number of mice.
Remember, biotic factors are the living things in an ecosystem.
- Q2 As the temperature fell, the size of the mouse population decreased. This could have been because the cold weather caused the temperature of the surroundings to fall below the body temperature of the mice. If that had happened the mice might have used up more energy maintaining their body temperature. This would have meant less energy was available for growth and reproduction, causing their population size to fall.
- Q3 As the mouse population size increased, there was more food for the owls and so the owl population grew. As the owl population increased, more mice were eaten and so the mice population began to fall. This meant there was less food for the owls, so their population decreased — and so this cycle continued.

Page 422 — Fact Recall Questions

- Q1 a) All organisms of one species in a habitat.
b) All the populations of different species in a habitat.
- Q2 Carrying capacity is the maximum stable population of a species that an ecosystem can support.
- Q3 Interspecific competition is when organisms of different species compete with each other for the same resources. Intraspecific competition is when organisms of the same species compete with each other for the same resources.

3. Investigating Populations

Page 425 — Application Questions

- Q1 a) She could place the quadrat on the ground at random locations across the field and count how much of the quadrat is covered by daisies. A square in the quadrat should be counted if it's more than half-covered by a particular species.
b) E.g. the student could divide the field into a grid and use a random number generator to select coordinates. The quadrat could then be placed at these coordinates and the number of daisies in each quadrat counted.
- Q2 The scientist could place a tape measure in a straight line from the sea to the top of the shore, to form a transect line. He could then place a quadrat at regular intervals along the transect, and count the number of limpets present in the quadrat, or the number of squares of the quadrat that contained limpets.

Page 427 — Application Question

- Q1 a) i) $\frac{19 \times 14}{3} = 88.67 = 89$ beetles
ii) $\frac{17 \times 21}{6} = 59.5 = 60$ beetles
b) No. E.g. the two samples were only taken a day apart, which may not be long enough for the marked beetles to mix back in with the population. Also, the beetles were marked with white paint which might have affected their chances of survival.

Page 429 — Application Questions

- Q1 The kite diagram shows that species A is present between 20 and 45 m from the road with a low percentage cover. It's also present between 80-140 m, and is most abundant between 130-140 m. Species B is present between 55-130 m from the road, and is most abundant between 60-85 m. Species C is present between 0-50 m from the road and is most abundant between 0-35 m.
The graph shows that soil salinity is high between 0-30 m from the road, falls sharply between 30-40 m, continues to fall until around 50 m and then remains low.
- Q2 Salinity is high in coastal areas, so plants that grow there must be able to tolerate/be adapted for these conditions. Species C is present between 0-50 m from the road boundary and this overlaps with where salinity is the highest (0-30 m). This suggests that species C is adapted to high salinity conditions and therefore is suited to growing in coastal areas.
- Q3 The data shows that at a high soil salinity there is an absence of species B, but this doesn't prove that salt spray from the road (and the resulting high salinity) is the cause. Species B might be absent for other reasons, e.g. because it is out-competed by species C.

Page 429 — Fact Recall Questions

- Q1 a) The number of individuals of one species in a particular area.
b) Where a particular species is within the area you're investigating.
- Q2 Frequency, which is the number of samples a species is recorded in. Percentage cover, which is how much of the area you're investigating is covered by a species.
- Q3 a) A square frame divided into a grid of 100 smaller squares by strings attached across the frame.
b) When quadrats are placed next to each other along transect to work out species frequency and percentage cover across an area/along the transect.
- Q4
- $$\text{Total population} = \frac{\text{Number caught in 1st sample} \times \text{Number caught in 2nd sample}}{\text{Number marked in 2nd sample}}$$

4. Succession

Page 433 — Application Questions

- Q1 Primary succession, because there is no soil present in 1800.
Remember, the key difference between primary and secondary succession is soil — it's present in secondary succession, but not in primary succession. If you have a good look at the graph you'll see that there's no soil moisture in 1800. That's a pretty good sign that there's no soil either.
- Q2 The dominant plant species would have been adapted to survive without much water/in a soil with low moisture content and fluctuating ground temperatures. They would have had seeds that could remain viable for long periods of time. They would have been species of small plants / they would not have been tree species.
There's a lot going on in the graph with all the different lines — and you could get something like this in the exam. Don't let the graph's complexity put you off though. Take your time and make sure you really understand what the graph is showing, and read the questions carefully so you pick out the right bits from the graph for your answers.
- Q3 E.g. the average length of time dominant plant seeds remained viable was relatively high between 1800 and 1860. This might have been because seeds that remained viable for a long time could lie dormant until conditions were favourable enough to germinate. Between about 1860 and 1880 the average length of time fell sharply, and then continued to fall more slowly until levelling off at around 1960. This may have been because the plants that were dominant between 1800 and 1860 were succeeded by other plant species which were better adapted to the changed conditions, e.g. a higher soil moisture content, so they no longer needed to be viable for long periods of time.
- Q4 Between 1800 and 1920 because there were no tree species present during this time, so there would have been more light / less shade cast by the trees.
- Q5 The soil moisture content is 0 between 1800 and 1820 because there is no soil. The soil moisture content increased gradually from 1820 until 1940 as the soil developed, then it increased more rapidly between 1940 and 2000 because the addition of decomposed organic material (plant material) helped to increase soil moisture content and the deeper soil was able to retain more water.

Page 433 — Fact Recall Questions

- Q1 Because ecosystems are constantly changing.
Q2 The process by which an ecosystem changes over time.
Q3 primary succession
Q4 E.g. marram grass, lichens, shrubs of the *Calligonum* genus.
Q5 The largest and most complex community of plants and animals that an ecosystem can support.

5. Conservation

Page 436 — Application Questions

- Q1 a) The areas of steppe in which succession was controlled by grazing and fire had the highest percentage cover of grasses compared to the control. The area of steppe controlled by fire also had the lowest percentage cover of trees. This would suggest that fire was the most effective method of controlling succession. Mowing was the least successful method. The area of steppe in which succession was controlled by mowing had the lowest percentage cover of grasses and the highest percentage cover of trees after the control.

If you're asked to compare the effectiveness of something like different conservation methods, always make sure you know what the aim of the method is. In this case, the nature reserve want to stop the forest developing (so they want a low percentage of trees) and to keep the grassland (so they want a high percentage cover of grass).

- b) E.g. Grazing is less dangerous than fire. / Grazing could cause less harm to other species than fire.

- Q2 There is a conflict between the needs of local people (for food and income) and the conservation aim of protecting the saiga. By educating local people about the importance of not hunting the saiga at unsustainable levels, and providing alternative sources of income (and food), this can contribute towards fewer saiga being killed and therefore the conservation of the species.

Although there is a conflict between conservation and local people here, overhunting the saiga is in no-one's interest — if the saiga is hunted to extinction, the local people exploiting it will lose the saiga as a form of food and income completely.

Page 436 — Fact Recall Questions

- Q1 The protection and management of species and habitats.
Q2 E.g. because there's often a conflict between human needs and conservation.
Q3 A managed fire is lit. After the fire, secondary succession will occur — the plant species that grow back first (pioneer species) are the species that are being conserved. Larger plant species will take longer to grow back and will be removed again the next time the area is burnt.
Q4 E.g. seed banks, captive breeding, fishing quotas, protected areas.

6. Conservation Evidence and Data

Page 439 — Application Questions

Q1 The average percentage cover of non-native species was greatest on the control grasslands and lowest on the grazed fields. The average percentage cover of native species was greatest on the grazed fields and lowest on the harrowed and seeded fields. Overall, non-native species make up a greater proportion of plants than native species on average across all field types.

- Q2 a) Grazing was the most successful method because it produced a larger proportion of native plants than harrowing and seeding, and the control, and a smaller proportion of non-native plants than harrowing and seeding, and the control.
b) Grazing worked because, e.g. the sheep prefer eating non-native to native plant species. / The non-native plants may have been less likely to grow back after grazing than the native plants. Seeding may not have worked because, e.g. the native plant seeds that were seeded on harrowed grassland were unable to compete with non-native seeds.

For questions asking you to suggest an answer, you need to use your common sense — you won't have been taught the answer.

- Q3 a) E.g. the first study used a much larger sample size (14 fields per method) than the second study (1 field per method). The larger sample size of the first investigation would reduce the likelihood that the results seen were due to chance, so the results are more likely to be valid in the first investigation compared to the second investigation.
b) A negative control was used. The control field had no method applied so therefore it was used to check that the independent variable (method applied) was the only thing affecting the dependent variable (average percentage cover) — this is the aim of a negative control.

Exam-style Questions — pages 441-442

- 1.1 E.g. they could set up a belt transect / place quadrats next to each other along a transect (**1 mark**). The transect could extend across the width of the field and the abundance of marsh marigolds recorded in each quadrat (**1 mark**).
1.2 They could count how many squares of each quadrat are covered by marsh marigolds by counting a square if it's more than half-covered (**1 mark**). The number of squares covered can be converted into a percentage (**1 mark**). Measuring percentage cover is a quick way to investigate the abundance of marsh marigolds as they wouldn't have to count all the individual marsh marigolds (**1 mark**).
1.3 The non-living features of an ecosystem (**1 mark**).
1.4 The graphs show that there is a positive correlation between the moisture of the soil and the abundance of marsh marigolds (**1 mark**). There is also a positive correlation between the soil pH and the abundance of marsh marigolds (**1 mark**). But you can't conclude from this data that all marsh marigolds will grow better in waterlogged ground, because the results are only taken from an investigation looking at one field (**1 mark**). The results don't show a causal relationship because it's not clear whether soil moisture or soil pH has the bigger effect on marsh marigold growth (**1 mark**). Also, there may be other factors involved that increase marsh marigold growth, e.g. fewer herbivores/organisms that consume plants near the stream (**1 mark**).

- 1.5 E.g. There is risk of flooding, so they should check the weather forecast before doing fieldwork to check for heavy rain which could cause the field to flood and result in conditions being unsuitable for fieldwork (**1 mark**). There is a risk of falling on the boggy ground, so they should wear suitable footwear such as wellies with good grip (**1 mark**). There are two marks available for this question — but it's not enough to just describe two risks, you've also got to come up with suggestions about how they can minimise each risk to get full marks.

- 2.1 He could use an appropriate method (e.g. pitfall trap) to capture and count a sample of centipedes (**1 mark**). The species Z centipedes caught should be marked in a harmless way and released back into their habitat (**1 mark**). After a week, the same method should be used to collect a second sample of centipedes from the same population (**1 mark**). The number of species Z centipedes in the second sample, and the number in the second sample that are marked, should be counted (**1 mark**). The population size can be estimated using the equation:

$$\text{Total population} = \frac{\text{Number caught in 2nd sample} \times \text{Number caught in 1st sample}}{\text{Number marked in 2nd sample}} \quad (\mathbf{1 \ mark})$$

Try to use the correct ecological terms in your answers, for example talk about centipede habitats (not 'places where centipedes live').

2.2 $\frac{10 \times 15}{8} = 18.75 = \mathbf{19 \ centipedes \ (1 \ mark)}$

- 3.1 E.g. over 50 years, succession might lead to plants growing tall enough to decrease light intensity to a level too low for the endangered species to grow (**1 mark**). The process of burning the heathland might also alter soil pH so it's less suitable for the endangered species (**1 mark**).
- 3.2 Between 1991 and mid-1992, the population size of heather and shrubs falls dramatically because they were burnt by the fire (**1 mark**), and the population size of insects falls because the loss of heather and shrubs causes them to lose their habitat/source of food (**1 mark**). From mid-1992 until 2000 the population size of heather and shrubs starts to rise as (secondary) succession occurs, and the growth of shrubs and heather provides a habitat/source of food for insects so their population also gradually increases (**1 mark**).
- 3.3 The first species to colonise an area during succession (**1 mark**).
- 3.4 E.g. herb species X was not present before 1992, before the fire, perhaps because the abiotic conditions weren't favourable (**1 mark**). Between 1992 and 1994 the population size of herb species X increased dramatically, so it must be better adapted than other species to the changed abiotic conditions created by the fire (**1 mark**). The increase in population size of herb species X slowed down and then levelled off between 1994 and 2003. It then decreased dramatically after 2003, perhaps because it was out-competed by another species or because it wasn't adapted to the changed abiotic conditions created by the increasing population size of other plant species (**1 mark**).
- 3.5 E.g. the graph only shows data over a nineteen year period after a fire and it might take longer than this for the insect population to recover / the population size of insects shows an upward trend, and this may continue after 2010 (**1 mark**). The graph only shows data for one year before the fire, and the insect population size was decreasing — so the data in 1990 might not be representative of the normal population size of insects (**1 mark**).

- 3.6 E.g. animals could be allowed to graze the land and eat the growing points of the shrubs and heather, stopping them from establishing themselves (**1 mark**). This would help to keep vegetation low and so increase the amount of light / reduce the amount of shade for the endangered plant species (**1 mark**). Controlling the soil pH would help to keep the soil acidic for the plant (**1 mark**).

Think about the conditions the plant needs to grow successfully in (which is given in the introduction to the question), and how these conditions can be brought about by different conservation methods.

Topic 8

Topic 8 — A: Mutations and Gene Expression

1. Mutations

Page 445 — Application Questions

Q1 a) Mutation A = a substitution mutation

The third base along is now C, not T.

Mutation B = a substitution mutation

The seventh base along is now C, not G.

Mutation C = a deletion mutation

The fourth base, C, has been deleted.

Mutation D = a duplication mutation

The first triplet, CTT, has been repeated.

b) Mutation A: Leu-His-Asp-Thr

Mutation B: Leu-His-His-Thr

Mutation C: Leu-Met-Ile

Mutation D: Leu-Leu-His-Asp-Thr

Q2 a) Mutation A is likely to have the least serious effect on the protein's structure. CTC still codes for Leu so the amino acid sequence/primary structure of the protein won't change.

This is an example of a silent mutation.

b) Mutation C is likely to have the most serious effect on the protein's structure as it is a frameshift mutation, which means all the amino acids coded for after/downstream of the mutation will be different.

Page 445 — Fact Recall Questions

Q1 A change to the base (nucleotide) sequence of DNA.

Q2 In a translocation mutation a sequence of bases is moved from one location in the genome to another.

Q3 The mutation may result in a change to the shape of the enzyme's active site. This may stop substrates from being able to bind to the active site, leaving the enzyme unable to catalyse the reaction.

Q4 A mutation that is passed on to an individual's offspring as a result of a mutation in their gametes.

Q5 No. The genetic code is degenerate/some amino acids are coded for by more than one triplet. This means that not all mutations will result in a change to the amino acid sequence of a protein.

2. Mutagenic Agents

Page 446 — Application Questions

Q1 2-aminopurine can pair with both thymine and cytosine, which could cause a substitution mutation in the new DNA during DNA replication.

Q2 Alkylating agents such as mustard gas can add an alkyl group to guanine, changing the structure so that it pairs with thymine (instead of cytosine), which may result in a change in the amino acid sequence of a polypeptide.

Page 446 — Fact Recall Questions

Q1 E.g. during DNA replication.

Q2 Something that causes an increase in the rate of mutations.

Q3 E.g. ultraviolet radiation / ionising radiation / base analogs / alkylating agents / some chemicals / some viruses.

3. Cancer

Page 450 — Application Questions

Q1 B. E.g. because the nuclei are larger and darker in B than in A, the cells are more irregularly shaped in B than A and the cells are denser in B than A (suggesting that they don't respond to growth regulating processes, and so divide more frequently than normal cells).

Q2 a) It slows cell division by producing a protein that stops cells dividing or causes them to self-destruct.
b) A mutation in p53 could inactivate the gene. The protein it codes for won't be produced. This will cause cells to start dividing uncontrollably, eventually leading to cancer.

Don't worry if you've never heard of the p53 gene — you can still answer the question. You just need to apply your knowledge of tumour suppressor genes to the p53 example. You're likely to get a lot of questions like this in the exam.

Q3 a) She has a malignant tumour as it has spread to other parts of her body.
b) E.g. HRT increases oestrogen levels in the body. Increased exposure to oestrogen over an extended period of time is thought to increase the risk of developing breast cancer.

Q4 Dichloroacetic acid causes hypomethylation of c-myc. Hypomethylation of proto-oncogenes like c-myc causes them to act as oncogenes, therefore increasing the production of proteins that encourage cell division. This stimulates cells to divide uncontrollably, which causes the formation of tumours.

Page 450 — Fact Recall Questions

Q1 A mutation that occurs in individual cells after fertilisation.

Q2 a tumour

Q3 Proto-oncogenes stimulate cell division by producing proteins that make cells divide.

Q4 A mutated proto-oncogene.

Q5 Any one from: e.g. a malignant tumour is cancerous, whereas a benign tumour is non-cancerous. / Malignant tumours grow rapidly, whereas benign tumours grow more slowly.

Q6 When tumour suppressor genes are hypermethylated, the genes are not transcribed, so the proteins they produce to slow cell division aren't made. This means that cells are able to divide uncontrollably by mitosis and tumours can develop.

Q7 Any one from: e.g. oestrogen can stimulate certain breast cells to divide and replicate. The more cell divisions that are taking place, the greater the chance of mutations occurring, and so the chance of cells becoming cancerous is increased. / If breast cells do become cancerous, oestrogen's ability to stimulate division could also help tumours to form quickly. / Exposure to oestrogen could introduce mutations directly into the DNA of certain breast cells, again increasing the chance of these cells becoming cancerous.

4. Interpreting Data on Cancer

Page 452 — Application Question

- Q1 a) The results show that the relative risk of breast cancer for women in California is higher for women who previously smoked or still smoke compared to those who have never smoked.
- b) No. E.g. just because there is a positive correlation between smoking and breast cancer, it doesn't mean that smoking causes breast cancer. The correlation may be due to chance, or there could be other risk factors involved that aren't considered here, such as genetic factors. Also, the study just looked at women in California so these results can't be applied to women in general.

Page 454 — Application Questions

- Q1 a) If a mutation only occurs in one of the RB1 alleles, the other one will still be able to produce a normal pRB tumour suppressor protein. This means that cell division can still be controlled. If a mutation occurs in both RB1 alleles, a normal pRB protein won't be produced and cells will start dividing uncontrollably. This could eventually lead to cancer.

b) E.g. it means that children who have inherited an RB1 mutation can be regularly screened, so that if further tumours do develop they can be diagnosed and treated earlier.

- Q2 a) If CD117 is present, then it may be possible to treat the cancer with Imatinib. If it is not present, then Imatinib will be ineffective and another course of treatment will be necessary.
- b) E.g. mutated tumour suppressor genes are inactivated. The protein they produce doesn't function. A drug which targets a mutated tumour suppressor gene would have to restore the function of the tumour suppressor protein, which could be difficult.

This is one of those 'suggest' questions where you're not expected to know the exact answer, but you should be able to come up with a suggestion using what you already know. A sensible answer, like the one above, will get you the marks in the exam.

5. Stem Cells

Page 457 — Application Questions

- Q1 unipotent
- Q2 a) pluripotent
b) In an embryo.
c) totipotent

Page 457 — Fact Recall Questions

- Q1 Unspecialised cells that can develop into other types of cell.
- Q2 In embryos and in some adult tissues.
- Q3 Totipotent stem cells can mature/develop into any type of body cell in an organism, multipotent stem cells can only differentiate into a few different types of cell and unipotent stem cells can only differentiate into one type of cell.
- Q4 Stem cells become specialised by only expressing certain genes and switching off others. Genes that are expressed get transcribed into mRNA, which is then translated into proteins. These proteins modify the cell. Changes to the cell produced by the proteins cause the cell to become specialised.
- Q5 a) a heart muscle cell
b) unipotent stem cells

6. Stem Cells in Medicine

Page 460 — Application Questions

- Q1 The bone marrow comes from a healthy donor who does not carry the mutation for sickle cell anaemia. As a result, the multipotent stem cells in the donor marrow will divide and differentiate to produce new, healthy red blood cells. These red blood cells won't sickle and so will function normally, curing the patient.
- Q2 a) Because the stem cells can be used to form new, specialised corneal cells to replace the damaged ones.
- b) E.g. any two from: there's a reduced risk of rejection as the donated cells are from the same patient. / There's no need to use embryonic stem cells, so avoids the ethical issues surrounding their use. / There's no need to wait for a donor cornea to become available.
- Q3 The plasmids could have the genes coding for transcription factors that are normally associated with pluripotent stem cells within their DNA. They could then be inserted into adult cells. The genes may then be passed into the adult cell's DNA, meaning that the cell is able to produce the transcription factors, and become pluripotent.
- Q4 Answer should include a discussion of the pros and cons of using embryonic stem cells in this particular investigation. E.g. pros: the embryonic stem cells are taken from donated embryos, which would otherwise be discarded. The treatment could improve the quality of the patients lives/ reduce the amount of medical care they require by giving them more movement.
- E.g. cons: the embryonic stem cells come from embryos which could have become a fetus if placed in the womb. There are other treatments also being developed, that use induced pluripotent stem cells, which means the patients could potentially be treated without needing embryonic stem cells to be used.
- If you're asked to discuss an issue such as this one, make sure you give both sides of the argument.

7. Regulation of Transcription and Translation

Page 463 — Application Questions

- Q1 Less of the transcription factor coded for by the MECP2 gene is produced, so it is unable to repress the transcription of other genes. These genes remain active which affects the normal functioning of nerve cells, leading to Rett syndrome. You don't need to know anything about Rett syndrome to answer this question because it's testing your knowledge of transcription. But you still need to make sure that you apply your knowledge to the disease in the question.
- Q2 siRNA could be produced that is complementary to the genes causing AMD. The siRNA and associated proteins would bind to the target mRNA and the proteins would cut up the mRNA into sections so it would no longer be translated.

Page 465 — Application Questions

- Q1 Any three from, e.g. temperature / the presence of other amino acids / the length of time the bacteria are left for / volume of culture / number of bacteria / amount of tryptophan added.
- Q2 a) In normal bacteria in the presence of tryptophan, the amount of target mRNA is reduced by about 70-fold. This is because when tryptophan is present it binds to the repressor, allowing it to bind to the target gene and reduce transcription. A reduction in transcription means much less mRNA is produced, and therefore much less tryptophan is produced.
- b) Bacteria do not need to produce more tryptophan if it's already present, so transcription stops to prevent the bacteria from wasting energy on producing something they don't need.
- Q3 The mutant bacteria produce a similar amount of tryptophan in both the presence and absence of tryptophan. This could be because, e.g. the mutation affected the DNA base sequence of the tryptophan repressor, so it can no longer bind to DNA even in the presence of tryptophan. This means it can't prevent transcription and so tryptophan is always produced.

Page 465 — Fact Recall Questions

- Q1 Protein molecules that control the transcription of genes.
- Q2 a) A transcription factor that increases the rate of transcription.
b) A transcription factor that decreases the rate of transcription.
- Q3 Because not all cell types have oestrogen receptors.
- Q4 Oestrogen binds to the oestrogen receptor (a transcription factor) to form an oestrogen-oestrogen receptor complex. The complex then moves from the cytoplasm to the nucleus and binds to the DNA near the start of the target gene.
- Q5 RNAi is where small, double-stranded RNA molecules stop mRNA from target genes being translated into proteins. The double-stranded siRNA molecule associates with several proteins in the cytoplasm and unwinds — one strand is selected and the other is degraded. The single strand of siRNA is fully complementary to the target mRNA and so binds to it. Its associated proteins cut the mRNA into fragments. The fragments are then moved to a processing body where they are degraded, and so the protein is not transcribed.
- Q6 A mammalian miRNA molecule is not fully complementary to the target mRNA base sequence, so it is less specific than siRNA (which is fully complementary to its target mRNA).

8. Epigenetic Control of Gene Expression

Page 468 — Application Questions

- Q1 The DNA structure at the methylated sites would be altered so that the transcriptional machinery cannot bind to the genes and so they would not be transcribed.
- Q2 E.g. different genotypes may have different responses to nutrient deprivation/environmental stress.
- Q3 Most epigenetic marks are removed between generations. It is possible that the methyl groups added in the parental plant during stress were removed during reproduction.

Page 468 — Fact Recall Questions

- Q1 E.g. methyl groups (on DNA) and acetyl groups (on histones).
- Q2 When acetyl groups are added to histones, the chromatin becomes less condensed, so transcriptional machinery can access the DNA and transcribe those genes.

9. Evaluating Data on Phenotypes

Page 470 — Application Question

- Q1 a) The mean difference in head circumference is approximately 0.5 cm for identical twins, 3 cm for non-identical siblings and 8.5 cm for unrelated individuals. So the mean difference in head circumference is much larger for unrelated individuals than for either identical twins or non-identical siblings.
- b) The data suggests that genetic factors have a larger effect on head circumference, because the mean difference in head circumference is much larger for unrelated individuals than for either identical twins or non-identical siblings. However, the mean difference for identical twins wasn't zero, so environmental factors appear to play some role.
- c) The mean difference in the number of steps taken is between 800 and 900 for all three sample groups. Identical twins and non-identical siblings show the lowest difference and unrelated individuals the highest but the margins are very small. This suggests that environmental factors play a more important role than genetic factors in determining activity level when measured by the number of steps taken per day.

Page 470 — Fact Recall Question

- Q1 They are genetically identical, so any differences in phenotype must be due to environmental factors.

Exam-style Questions — pages 472-473

- 1.1 deletion (**1 mark**)
1.2 mRNA codons: ACG GUU GUU GUG AGC (**1 mark**)
amino acids: Threonine, Valine, Valine, Valine, Serine (**1 mark**)
It's always a good idea to show your working for questions like this, even if it doesn't say to in the question, as you might pick up a mark even if you get the amino acids wrong.
- 1.3 It is an activator. The results show that both the normal and the mutant bacteria produce the transcription factor (7.9 and 7.7 arbitrary units) (**1 mark**). But the mutant bacteria produce much less antibiotic mRNA (0.9 arbitrary units compared to 8.2) and much less of the antibiotic itself (the blue-colour of the antibiotic can't be seen around the mutant bacteria) (**1 mark**). This means that the mutation must affect the transcription of mRNA (**1 mark**). This suggests the mutant bacteria have a faulty transcription factor protein which can't bind to the start of the target gene and so can't activate transcription (**1 mark**).
The introduction to question 1 says that the bacteria produce a blue-coloured antibiotic. Figure 2 shows that mutant bacteria don't produce the blue colour, which suggests they don't produce the antibiotic.
- 2.1 The APC gene must be the tumour suppressor gene. It slows cell division by preventing β -catenin from carrying out its function (**1 mark**). β -catenin activates genes needed for cell division, so the β -catenin gene must be the proto-oncogene (**1 mark**).

- 2.2 E.g. a mutation in the β -catenin gene could cause it to become overactive and stimulate the cell to divide uncontrollably, causing cancer (**1 mark**). A mutation in the APC gene could prevent the protein from carrying out its function of destroying β -catenin, so the cell would be stimulated to divide uncontrollably (**1 mark**).
- 2.3 A deletion of one or more bases will affect the number of bases present, causing a shift in all the base triplets that follow (**1 mark**) and this could cause a change in the amino acid sequence/primary structure of the protein (**1 mark**). This could change the tertiary structure of the protein and prevent it from functioning (**1 mark**).
- 2.4 Any one from: e.g. it could allow you to have regular screening/tests to help doctors diagnose colon cancer in its early stages. / It may affect the treatment you are given if and when you develop colon cancer. (**1 mark for any sensible answer.**)
- 3.1 As the plant cells become more specialised, the relative concentration of alkaloid in the cells increases (**1 mark**).
- 3.2 $\frac{1.85 - 0.45}{0.45} \times 100 = 311\%$ (accept 300 to 322%) (**1 mark**)
- 3.3 E.g. the auxins are needed to quickly produce a large number of cells early on in the tissue culture (**1 mark**), but alkaloid production is much higher in specialised cells, so auxins are later removed because they reduce cell specialisation (**1 mark**).
- 3.4 E.g. cells become specialised by expressing certain genes and switching off others (**1 mark**). Genes that are expressed get transcribed into mRNA and then translated into proteins (**1 mark**). The proteins then modify the cell, causing it to become specialised (**1 mark**). If auxins alter the genes that get expressed, the cell will transcribe and translate different proteins (**1 mark**). These proteins could then modify the cell in a different way, changing the type of cell it specialises into (**1 mark**).

Topic 8 — B: Genome Projects and Gene Technologies

1. Genome Projects

Page 475 — Fact Recall Questions

- Q1 To determine the complete set of genetic material in an organism.
- Q2 a) The proteome is all the proteins that can be made by an organism.
b) E.g. you can use it to identify the protein antigens on the surface of disease-causing bacteria and viruses, which can help in the development of vaccines to prevent the disease.
- Q3 More complex organisms contain large sections of non-coding DNA and also complex regulatory genes, which determine when the genes that code for particular proteins should be switched on and off. This makes it more difficult to translate their genome into their proteome, because it's hard to find the bits that code for proteins among the non-coding and regulatory DNA.
- Q4 In the past, many sequencing methods were labour-intensive, expensive and could only be done on a small scale. Now these techniques are often automated, more cost-effective and can be done on a large scale.

2. Making DNA Fragments

Page 478 — Application Questions

- Q1 reverse transcriptase
- Q2 There is a *Bam*H site on the left hand side of the fragment and an *Eco*R site towards the right hand side. The DNA sample could be incubated with *Bam*H and *Eco*R, which would cut the DNA via a hydrolysis reaction at these sites. This is because the shape of each recognition sequence is complementary to each enzyme's active site.

Page 478 — Fact Recall Questions

- Q1 Recombinant DNA technology involves transferring a fragment of DNA from one organism to another.
- Q2 It is possible to transfer DNA to a recipient organism of a different species because the genetic code and transcription and translation mechanisms are universal.
- Q3 a) complementary DNA / a DNA copy of an mRNA molecule
b) mRNA is isolated from cells, then mixed with free DNA nucleotides and reverse transcriptase. The reverse transcriptase uses the mRNA as a template to synthesise new strands of cDNA.
c) E.g. mRNA is often easier to obtain than a DNA fragment containing the target gene. / There are generally more mRNA versions of a gene than DNA versions of a gene in a cell. / Only the exons are present in mRNA.
- Q4 A sequence of DNA that consists of antiparallel base pairs/base pairs that read the same in opposite directions.
- Q5 Small tails of unpaired bases at the end of a DNA fragment. They can be used to bind/anneal the DNA fragment to another piece of DNA that has sticky ends with complementary sequences.
- Q6 The sequence that is required is designed. The first nucleotide in the sequence is then fixed to some sort of support. Nucleotides are added step by step in the correct order, in a cycle of processes that includes adding protecting groups. Short sections of DNA called oligonucleotides, roughly 20 nucleotides long, are produced. Once these are complete, they are broken off from the support and all the protecting groups are removed. The oligonucleotides can then be joined together to make longer DNA fragments.

3. Amplifying DNA Fragments

Pages 482-483 — Application Questions

- Q1 a) Vector DNA was cut with the same restriction endonucleases as the DNA fragment, so complementary sticky ends were produced. The DNA fragment and cut vector were mixed with ligase and the pieces were joined together to form the recombinant DNA. A marker gene must also be present in the recombinant DNA, so may have been inserted at the same time as the DNA fragment or existed in the vector DNA already.
In the exam, put down as much information as you can. Here there are a few details on how the DNA fragment was made and how the marker gene got in there, as they all form part of the recombinant DNA.
b) It's likely he put a marker gene for resistance to penicillin in as part of the recombinant DNA. He grew the plates on agar containing penicillin so he could identify which colonies contained transformed cells (cells with the target gene in).

Q2 a) top strand = CGTA, bottom strand = GGT A

$$b) 2 \times 2 \times 2 \times 2 \times 2 \times 2 = 128$$

Remember, you start with two single strands of DNA.

The amount of DNA then doubles with each PCR cycle.

- Q3 a) The plasmids are vectors — they're used to transfer the target gene into the host cells/*E.coli*.
b) The ampicillin resistance gene is a marker gene. It means that only *E. coli* containing the plasmid will grow on the agar (which contains ampicillin).
c) *E.coli* that have taken up plasmids containing the target gene will be white/colourless because the target gene has disrupted the LacZ α gene in the bacterial plasmids. This means the LacZ α gene won't have produced the correct protein, so the *E.coli* won't have been able to produce β -galactosidase and therefore won't have been able to break down X-gal into a blue pigment. *E.coli* containing plasmids without the target gene will be blue because they will contain both the LacZ α and LacZ Ω genes — this will enable them to produce functional β -galactosidase and therefore breakdown X-gal into a blue pigment.

Page 483 — Fact Recall Questions

- Q1 In *in vivo* cloning, gene copies are made inside a living organism. In *in vitro* cloning, gene copies are made outside of a living organism (using PCR).
Q2 A vector is something that's used to transfer DNA into a cell.
Q3 E.g. a plasmid / bacteriophage
Q4 DNA ligase is used to join the sticky ends of the DNA fragment containing the target gene to the sticky ends of the vector DNA.
Q5 A cell into which the target gene/recombinant DNA is transferred.
Q6 That it has taken up the vector containing the target gene.
Q7 Not all cells will take up the vector — the ones that do need to be identified so they can be allowed to grow and produce lots of copies of the cloned gene.
Q8 Identifying transformed cells.
Q9 Promoter and terminator regions
Q10 The polymerase chain reaction (PCR).
Q11 a) The DNA sample, free nucleotides, primers and DNA polymerase.
b) The DNA mixture is heated to 95 °C to break the hydrogen bonds between the two strands of DNA. The mixture is then cooled to between 50 and 65 °C so that the primers can bind (anneal) to the strands. The reaction mixture is then heated to 72 °C, so DNA polymerase can work. The DNA polymerase lines up free DNA nucleotides alongside each template strand. Specific base pairing means new complementary strands are formed. Two new copies of the fragment of DNA are formed and one cycle of PCR is complete. The cycle then starts again.

4. Recombinant DNA Technology

Page 486 — Application Questions

- Q1 A DNA fragment containing the resistance gene could be made using reverse transcriptase, PCR or cut out using restriction endonucleases. The DNA fragment could be inserted into a plasmid vector which could then be added to a bacterium. The bacterium could then be used as a vector to get the gene into the soybean plant cells.
Q2 Fields of the soybean crop could be sprayed with the herbicide, killing the weeds but not the crop. This could increase the yield from the field.
Q3 E.g. if the transformed soybean crop interbreed with wild plants it could possibly result in 'superweeds' — weeds that are resistant to a herbicide. / Farmers might plant only this soybean crop, which could make the whole crop vulnerable to the same disease because the plants are genetically identical/biodiversity in the area is reduced. / This one large agricultural company could end up controlling soybean production, forcing smaller companies out of business. These are all good answers, but as the question specifically talks about herbicide resistance, the superweed concern is probably the best answer to give as it's the most specific to the context you're given.

5. Gene Therapy

Page 487 — Fact Recall Questions

- Q1 Gene therapy involves altering the defective genes (mutated alleles) inside cells by inserting a DNA fragment into the original DNA.
Q2 Somatic therapy involves altering the alleles in body cells, particularly the cells that are most affected by the disorder, whereas germ line therapy involves altering the alleles in the sex cells.

6. Gene Probes and Medical Diagnosis

Page 490 — Application Questions

- Q1 A DNA microarray should be used — microscopic spots of DNA probes for different genetic mutations (different genetic disorders) are attached to a glass slide in rows. A fluorescently labelled sample of the child's DNA is washed over the array. The array is washed and visualised under UV light. Any labelled DNA attached to a probe will fluoresce, identifying any mutations in the child's DNA and so which genetic disorder they have.
Q2 They may undergo genetic counselling to help provide them with information on the treatment available for their child. They might also have counselling to help them understand the chances of them having another child with the recessive disorder. Genetic counselling will provide them with unbiased advice on the possibility of having IVF and screening their embryos for the alleles.

7. Genetic Fingerprinting

Page 494 — Application Questions

- Q1 Some parts of an organism's genome consist of variable number tandem repeats (VNTRs). These repeated sequences occur in lots of places in the genome and the number of times these sequences are repeated differs from organism to organism. Genetic fingerprinting looks at the number of times some of these sequences are repeated at different loci in an individual's genome.
- Q2 a) To make many copies of the areas of DNA that contain the repeated sequences. / To add fluorescent tags to the DNA so it can be seen under UV light.
b) To separate the fragments of DNA by length, producing a genetic fingerprint.
These techniques can be used for lots of different reasons in gene technology. So make sure you clearly understand what each one does, then you should be able to work out why it's been used in any situation you're given.
- Q3 No, the woman does not appear to be the diplomat's daughter. Only one band is found in the same position for both the woman and the diplomat. You would expect more than this if he was her father.

Exam-style Questions — pages 496-497

- 1.1 Any two from, e.g. they may have been concerned that the crop would encourage farmers to plant monocultures, reducing biodiversity (**1 mark**). / They may have been concerned about the possibility of 'superweeds' — weeds that are resistant to herbicides because they've bred with genetically engineered herbicide-resistant crops (**1 mark**). / They may have been concerned that organic farmers nearby will have their crops contaminated by wind-blown seeds from the genetically modified crops (**1 mark**).
- 1.2 A DNA probe is a short strand of DNA with a base sequence that is complementary to a target sequence (**1 mark**).
- 1.3 A sample of DNA containing the resistance gene is digested using restriction enzymes and the digested fragments are separated by electrophoresis (**1 mark**). The separated fragments are then transferred to a nylon membrane and incubated with a fluorescently labelled DNA probe (**1 mark**). The probe will hybridise to any DNA fragment that contains a complementary DNA sequence (**1 mark**). The membrane is then exposed to UV light / X-ray film, so that the band the probe has attached to can be visualised (**1 mark**).
- 1.4 Colonies were added to the plate containing penicillin to identify the transformed cells (those that have taken up the recombinant DNA containing the target allele) (**1 mark**). A marker gene/genetic marker for penicillin resistance was added to the recombinant DNA so that only transformed cells will grow on plates containing penicillin, allowing them to be identified (**1 mark**). Colonies of bacteria were also added to a standard agar plate as a control to show that all the colonies grew in the absence of penicillin (**1 mark**).
- 1.5 Bacteria A is a control to make sure nothing in the host cells on their own makes them resistant to penicillin/ to make sure the penicillin is working (**1 mark**).
- 1.6 Any one of 1, 3, 5 or 7. These grew on the penicillin plate, and so must be transformed cells/contain the recombinant DNA with the allele of interest and the marker gene/genetic marker for penicillin-resistance (**1 mark**).
- 2.1 PCR is used to make many copies of the areas of the DNA that contain the VNTRs in each DNA sample (**1 mark**). A fluorescent tag is added to all the DNA (to allow it to be seen under UV light) (**1 mark**). The PCR mix from each sample is separated using gel electrophoresis (**1 mark**). Shorter DNA fragments move faster and travel further through the gel, so the DNA fragments separate according to length with longer pieces nearer the negative end (**1 mark**). The gel is placed under UV light to see the bands produced for each sample — these are the genetic fingerprints (**1 mark**).
- 2.2 Genetic fingerprint technology involves comparing the number of times repetitive, non-coding base sequences (**1 mark**) are repeated at a number of different, specific places (loci) in a genome (**1 mark**). The probability of two individuals having the same genetic fingerprint is very low because the chance of two individuals having the same number of sequence repeats at each locus tested is very low (**1 mark**).
- 2.3 Yes. The genetic fingerprint of the stolen horse and genetic fingerprint of horse 3 have exactly the same band pattern, so the DNA that produced both genetic fingerprints must have come from the same horse (**1 mark**).
- 2.4 Horse 4 and the stolen horse have 6 matching bands (**1 mark**), which suggests they must be closely related in some way (**1 mark**). The stolen horse had previously been sent to the farm for breeding purposes, so it seems likely that horse 4 is a child of the stolen horse (**1 mark**). Offspring get 50% of their DNA from each parent, so roughly 50% of bands will match between a parent and child in a genetic fingerprint.
- 2.5 E.g. determining genetic relationships / determining genetic variability within a population / medical diagnosis / animal and plant breeding (**1 mark**).
- 3.1 E.g. they could use restriction endonuclease enzymes (**1 mark**) to cut the DNA at specific palindromic recognition sequences (**1 mark**) / They could use a gene machine (**1 mark**) to create fragments of the required sequence from scratch without the need for a pre-existing DNA template (**1 mark**).
- 3.2 They are important in *in vivo* cloning as complementary sticky ends are required to anneal (bind) the target DNA fragment and vector DNA together (**1 mark**).
- 3.3 Any six from: the vector DNA is cut using the same restriction enzyme to produce complementary sticky ends (**1 mark**). The DNA fragment containing the target gene and vector DNA are mixed together with DNA ligase (**1 mark**), which joins the sticky ends together creating recombinant DNA (**1 mark**). Marker genes are inserted into the vector at the same time as the DNA fragment (**1 mark**). The vector with the recombinant DNA and the fluorescent marker gene is then used to transfer the gene into host cells (**1 mark**). The host cells are grown on agar plates to produce colonies of cloned cells (**1 mark**). If the agar plate is placed under UV light, only colonies of transformed cells will fluoresce because only these cells will contain the marker gene (**1 mark**). Identified transformed cells are allowed to grow more producing lots of copies of the cloned gene (**1 mark**).

Exam Help

Page 500 — Exam-style Question

Q1 21-25 marks:

The answer includes material from a variety of different topic areas and clearly shows its link to the question title. No irrelevant material is included. The answer includes a range of detailed and accurate biological facts that are all of A-level standard. No incorrect material is included. Appropriate scientific terminology is used. Explanations are clear and the overall essay is very well written.
(To get top marks, evidence of wider reading beyond the specification must be shown.)

16-20 marks:

The answer includes material from several relevant topic areas and links these to the question title. An irrelevant topic may be included. The answer includes a range of biological facts that are accurate and of A-level standard but may sometimes be lacking in detail. There may be one significant error in the scientific content. Appropriate scientific terminology is used. Explanations are clear.

11-15 marks:

The answer includes material from several relevant topic areas but doesn't link them to the question title. More than one irrelevant topic may be included. The biological facts included in the answer are mostly correct and of A-level standard but material is lacking in detail. There may be a few significant errors in the scientific content.
Appropriate scientific terminology is usually used.
Explanations are usually clear.

6-10 marks:

The answer includes material from one or two relevant topic areas but doesn't link them to the question title.
Several irrelevant topic areas may be included.
Some A-level content may be included but it will be lacking in detail and may contain several significant scientific errors. There may be limited use of scientific terminology.
Explanations lack clarity.

1-5 marks:

The answer includes material that is only vaguely linked to the question title. Material is presented as a series of facts. Most of the material is irrelevant. The content is below A-level standard and contains a large number of scientific errors. Scientific terminology is not used or is below A-level standard. Explanations are poor or absent.

0 marks:

Nothing relevant is included in the answer or nothing has been written.

Here are some topic areas you might write about:

- the importance of hydrogen ions in the redox reactions of photosynthesis and respiration;
- how the concentration of hydrogen ions determines the pH of solutions and how maintaining the pH of the blood is important for many enzyme-controlled metabolic reactions;
- the use of iron ions in haemoglobin to transport oxygen around the body;
- how the movement of sodium and potassium ions creates action potentials, allowing nervous communication;
- how calcium ions are involved in the transmission of action potentials across synapses;
- the use of sodium ions in the co-transport of glucose and amino acids into cells;

- the use of phosphate ions in the production of ATP, and the importance of ATP in storing and releasing energy for the cell;
- the use of phosphate ions in producing DNA and RNA nucleotides and the importance of these molecules as the carriers of genetic information.

This is not a full list of all the topic areas you could write about — it's just to give you an idea. Remember, you should aim to write about at least five of these topic areas. Whatever topic areas you include, you must relate them to the essay title — so in this case, don't just write about inorganic ions, make it really clear how inorganic ions are important to living organisms.

Glossary

A

Abiotic condition

A non-living feature of an ecosystem.

Abundance

The number of individuals of one species in a particular area (i.e. population size).

Accurate result

A result that is really close to the true answer.

Acetylation

Attachment of an acetyl group to something (e.g. histones).

Acetylcholine (ACh)

A type of neurotransmitter that binds to cholinergic receptors.

Acetyl coenzyme A (AcetylCoA)

A type of coenzyme involved in respiration. It transfers acetate from one molecule to another.

Acquired mutation

A mutation you develop during your lifetime.

Actin

The thin myofilament protein in muscle fibres.

Actin-myosin cross bridge

The bond formed when a myosin head binds to an actin filament.

Activation energy

The energy that needs to be supplied before a chemical reaction will start.

Activator

A transcription factor that increases the rate of transcription.

Active site

The part of an enzyme where a substrate molecule binds.

Active transport

Movement of molecules and ions across plasma membranes, usually against a concentration gradient. Requires energy.

Adaptation

A characteristic that increases an organism's chances of survival and reproduction, e.g. antibiotic-resistance.

ADP (adenosine diphosphate)

A molecule made up of adenine, a ribose sugar and two phosphate groups. ATP is synthesised from ADP and a phosphate group.

Adrenaline

A hormone secreted from the adrenal glands that has many effects, including increasing the blood glucose concentration.

Affinity for oxygen

The tendency a molecule has to bind with oxygen.

Agglutination

The clumping together of cells, e.g. pathogens, red blood cells.

AIDS (acquired immunodeficiency syndrome)

A condition caused by HIV, in which the immune system deteriorates and eventually fails.

Allele

One or more alternative versions of the same gene.

Allele frequency

How often an allele occurs in a population.

Allopatric speciation

Where speciation occurs as a result of geographic isolation.

Alveolus

A microscopic air sac in the lungs where gas exchange occurs.

Amino acid

A monomer of proteins.

Ammonification

The process in which nitrogen compounds from dead organisms or waste material are turned into ammonium compounds by saprobionts.

Anomalous result

A measurement that falls outside the range of values you'd expect or any pattern you already have.

Antibiotic

A medicine that is designed to kill or inhibit the growth of bacteria (or sometimes fungi).

Antibiotic resistance

When bacteria are able to survive in the presence of antibiotics.

Antibody

A protein produced by B-cells in response to the presence of a pathogen.

Antidiuretic hormone (ADH)

A hormone that regulates the water potential of the blood by controlling the permeability of the cells of the distal convoluted tubule and the collecting duct in the kidney.

Antigen

A molecule (usually a protein) that can trigger an immune response.

Antigenic variation

Where pathogens change their antigens.

Antigen-presenting cell

An immune system cell that processes and presents antigens on its surface to activate other immune system cells.

Antimicrobial substance

A substance designed to kill microorganisms, e.g. an antibiotic, antiseptic or disinfectant.

Artifact (microscope)

Something you can see on a microscope slide that isn't part of the specimen you're looking at, e.g. an air bubble.

Arteriole

A blood vessel that branches off an artery.

Aseptic technique

A technique used to prevent the unwanted growth or transfer of microorganisms.

Atheroma

A fibrous plaque caused by the build up and hardening of white blood cells, lipids and connective tissue.

ATP (adenosine triphosphate)

A molecule made up of adenine, a ribose sugar and three phosphate groups. It is the immediate source of energy in a cell.

ATP hydrolase

An enzyme which catalyses the hydrolysis of ATP into ADP and P_i.

ATP-phosphocreatine (PCr) system

A system that generates ATP very quickly by phosphorylating ADP using a phosphate group from phosphocreatine.

ATP synthase

An enzyme which catalyses the synthesis of ATP from ADP and P_i.

Atrioventricular node (AVN)

A group of cells in the heart wall that is responsible for passing waves of electrical activity from the SAN on to the bundle of His.

Atrioventricular valve (AV)

A valve in the heart linking the atria to the ventricles.

Attachment protein (virus)

A protein on the surface of a virus that lets the virus cling onto a suitable host cell.

Autonomic nervous system

A division of the peripheral nervous system that controls unconscious activities, e.g. heart rate.

Autoradiography

A technique that reveals the location of radioactive tracers.

Autosomal linkage

When two genes are located on the same autosome and are inherited by the offspring together.

Autosome

A chromosome that isn't a sex chromosome.

B**Base**

A nitrogen-containing molecule that forms part of a DNA nucleotide.

B-cell

A type of white blood cell involved in the immune response. It produces antibodies.

Benedict's test

A biochemical test for the presence of sugars.

Benign tumour

A non-cancerous tumour.

Bias

When someone intentionally, or unintentionally, favours a particular result.

Bile salt

A type of salt produced by the liver to aid the digestion of lipids.

Binary fission

The process by which prokaryotic cells replicate.

Binomial system

The system used in classification for naming organisms using a two-part Latin name.

Biodiversity

The variety of living organisms in an area.

Biomass

The mass of living material in an organism.

Biotic condition

A living feature of an ecosystem.

Biuret test

A biochemical test for the presence of polypeptides and proteins.

Bohr effect

An effect by which an increase of carbon dioxide in the blood results in a reduction of haemoglobin's affinity for oxygen.

Bundle of His

A group of muscle fibres in the heart, responsible for conducting waves of electrical activity from the AVN to the Purkyne tissue.

C**Cancer**

A tumour that invades surrounding tissue.

Capillary bed

A network of capillaries.

Capsid

The protein coat surrounding a virus's genetic material.

Capsule (cell)

A layer of secreted slime surrounding some prokaryotic cells.

Cardiac cycle

An ongoing sequence of contraction and relaxation of the atria and ventricles that keeps blood continuously circulating the body.

Cardiac output

The volume of blood pumped by the heart per minute (measured in cm³ per minute).

Cardiomyocyte

A heart muscle cell.

Cardiovascular disease

Any disease associated with the heart and blood vessels.

Carrier

A person carrying an allele that is not expressed in their phenotype, but that can be passed on to their offspring.

Carrier protein

A protein in a cell membrane that allows the facilitated diffusion of large molecules.

Carrying capacity

The maximum stable population size of a species that an ecosystem can support.

Catalyst

A chemical that speeds up a chemical reaction without being used up itself.

Causal relationship

Where a change in one variable causes a change in the other.

cDNA (complementary DNA)

A DNA copy of mRNA made using reverse transcriptase.

Cell cycle

The process that all body cells from multicellular organisms use to grow and divide.

| | | |
|--|---|--|
| Cell fractionation | Cholinergic synapse | Condensation reaction |
| A method that separates the organelles in a cell. | A synapse that uses the neurotransmitter acetylcholine. | A reaction that releases a molecule of water when it links molecules together. |
| Cell-surface membrane | Chromatid | Cone (eye) |
| The membrane found on the surface of animal cells (and just inside the cell wall of other cells). Regulates the movement of substances into and out of the cell. | One 'arm' of a double stranded chromosome. | A photoreceptor cell found in the eye that gives information in colour. |
| Cellular immune response | Chromosome | Conservation |
| The immune response that involves T-cells and the other immune system cells they interact with, e.g. phagocytes. | A thread like structure made up of one long DNA molecule. | The protection and management of species and habitats (ecosystems) in a sustainable way. |
| Cellulose | Chromosome non-disjunction | Continuous data |
| A polysaccharide made of long, unbranched chains of β -glucose. | Failure of the chromosomes to separate properly during meiosis or mitosis. | Data that can take any value in a range. |
| Cell wall | Classification | Control group |
| The outermost cell layer found in plant, algal and fungal cells. | The act of arranging organisms into groups. | A group in a study that is treated in exactly the same way as the experimental group, apart from the factor you're investigating. |
| Centromere | Climax community | Control variable |
| The point at which two strands of a chromosome are joined together. | The largest and most complex community of plants and animals an ecosystem can support. | A variable you keep constant throughout an experiment. |
| Channel protein | Codominant allele | Coordinator |
| A protein that forms a pore in a cell membrane and allows the facilitated diffusion of charged particles. | An allele whose characteristic appears together with another allele in the phenotype because neither allele is recessive. | Part of the nervous system (e.g. the CNS) which formulates an appropriate response to a stimulus before sending impulses to an effector. |
| Chemical mediator | Codon | Coronary artery |
| A chemical messenger that acts locally (i.e. on nearby cells). | A base triplet (three nucleotides) in DNA or mRNA that codes for an amino acid. | An artery supplying the heart muscle with blood. |
| Chemiosmosis | Coenzyme | Coronary heart disease |
| The process of electrons flowing down the electron transport chain and creating a proton gradient across a membrane to drive ATP synthesis. | A molecule that aids the function of an enzyme. They work by transferring a chemical group from one molecule to another. | When the coronary arteries have lots of atheromas in them, which restricts blood flow to the heart. |
| Chlorophyll | Community | Correlation |
| A photosynthetic pigment found in chloroplasts. There are different types of this pigment, e.g. chlorophyll a. | All the populations of different species in a habitat. | A relationship between two variables. |
| Chloroplast | Compensation point | Co-transporter |
| An organelle present in plant and algal cells where photosynthesis occurs. | The point at which the rate of photosynthesis in a plant exactly matches its rate of respiration. | A type of carrier protein that binds two molecules at the same time. |
| Choice chamber | Competitive inhibitor | Counter-current system (fish) |
| A container with different compartments that can be used to investigate how animals respond to different environmental conditions. | A molecule that has a similar shape to a substrate and blocks an enzyme's active site. | The system in which blood flows in one direction and water flows in the opposite direction across the gills of a fish. |
| Cholesterol | Complementary base pairing | Courtship behaviour |
| A type of lipid present in cell membranes (except bacterial cell membranes). | Hydrogen bonding between specific pairs of bases on opposing polynucleotide strands. | Behaviour carried out by organisms to attract a mate of the right species. |
| | | Crossing over |
| | | When chromatids twist around each other and bits of them swap over during meiosis. |

Cytokinesis

The division of the cytoplasm during eukaryotic cell division.

Cytoplasm

A gel-like substance where most of the chemical reactions in a cell happen.

Cytotoxic T-cell

A T-cell that kills abnormal or foreign cells.

D

Dehydrogenase

An enzyme that transfers hydrogen and electrons from one molecule to another.

Denatured

The point at which an enzyme no longer functions as a catalyst.

Denitrification

The process in which nitrates in the soil are converted into nitrogen gas by denitrifying bacteria.

Deoxyribose

The pentose sugar in DNA.

Dependent variable

The variable you measure in an experiment.

Depolarisation

A decrease in the potential difference across a cell's membrane, making it less negative (i.e. more positive) than the resting potential.

Diabetes mellitus (Type I)

A condition in which blood glucose concentration can't be controlled properly because the body doesn't produce any insulin.

Diabetes mellitus (Type II)

A condition in which blood glucose concentration can't be controlled properly because the body doesn't produce enough insulin or the body's cells don't respond properly to insulin.

Dicotyledonous plant

A type of flowering plant, e.g. non-woody plants, bushes and trees.

Differential reproductive success

The fact that in any population, some individuals are more likely to survive and reproduce than others.

Diffusion (simple)

Net movement of particles from an area of higher concentration to an area of lower concentration.

Digestion

The process of breaking down food into substances that can be used by the body.

Dihybrid inheritance

The inheritance of two characteristics, which are controlled by different genes.

Dipeptidase

An endopeptidase enzyme that hydrolyses peptide bonds within a protein.

Dipeptide

A molecule formed from two amino acids.

Diploid

When a cell contains two copies of each chromosome.

Directional selection

Where individuals with alleles for a single extreme phenotype are more likely to survive, reproduce and pass on their alleles.

Disaccharidase

An enzyme that catalyses the hydrolysis of disaccharides.

Disaccharide

A molecule formed from two monosaccharides.

Discrete data

Numerical data that can only take certain values in a range.

Disruptive selection

Where individuals with alleles for phenotypes at the extreme ends of a range are more likely to survive, reproduce and pass on their alleles.

Distribution

Where a particular species is within an area being investigated.

DNA (deoxyribonucleic acid)

The molecule in cells that stores genetic information.

DNA helicase

An enzyme that breaks the hydrogen bonds between two polynucleotide DNA strands during DNA replication.

DNA polymerase

An enzyme that joins together the nucleotides on a new strand of DNA during DNA replication.

DNA probe

A short single strand of DNA that has a complementary base sequence to part of a target gene.

DNA sequencing

A technique used to determine the order of bases in a section of DNA.

Dominant allele

An allele whose characteristic appears in the phenotype even when there's only one copy.

Double-helix

The structure of a DNA molecule — two separate strands wound together in a spiral.

E

Ecosystem

All the organisms living in a community plus all the non-living (abiotic) conditions in the area in which they live.

Effector

A cell that brings about a response to a stimulus, to produce an effect.

Electrochemical gradient

A concentration gradient of ions.

Electron transport chain

A chain of proteins down which excited electrons flow.

Emulsion test

A biochemical test for the presence of lipids.

Endopeptidase

An enzyme that hydrolyses peptide bonds within a protein.

Endoplasmic reticulum

A system of membranes enclosing a fluid-filled space. Involved with lipid and protein processing.

Endothelium

The inner lining of a blood vessel.

E

Enzyme

A protein that speeds up the rate of chemical reactions.

Enzyme-substrate complex

The intermediate formed when a substrate molecule binds to the active site of an enzyme.

**Epigenetic control
(of gene expression)**

The attachment or removal of chemical groups to or from DNA or histone proteins, which determines whether a gene is switched on or off.

Epistasis

When an allele of one gene masks (blocks) the expression of the alleles of other genes.

Eukaryote

Organism made up of a cell (or cells) containing a nucleus, e.g. animals, plants, algae and fungi.

Eutrophication

The process whereby nutrients build up in water, leading to the growth of large quantities of algae. This results in the death of plants, and the decomposition of dead plant matter causes the oxygen content of the water to fall, killing aquatic organisms.

Evolution

The gradual change in a species over time or the change in allele frequency in a population over time.

Exchange organ

An organ (e.g. the lungs) specialised to exchange substances.

Exocytosis

The process by which a cell secretes substances using vesicles.

Exon

A section of DNA within a gene that codes for amino acids.

Exopeptidase

An enzyme that hydrolyses the peptide bonds at the end of proteins to remove single amino acids.

Expiration

Breathing out.

Extracellular digestion

When food is broken down (digested) outside a cell. Saprobionts feed using extracellular digestion.

F

Facilitated diffusion

The diffusion of particles through carrier proteins or channel proteins in the plasma membrane.

FAD

A type of coenzyme involved in respiration. It transfers hydrogen from one molecule to another.

Fast twitch muscle fibre

A muscle fibre that contracts very quickly but also gets tired quickly.

Fertilisation

When a haploid sperm fuses with a haploid egg to generate a diploid zygote.

Flagellum

A long, hair-like structure that rotates to move a cell.

Fluid mosaic model

Model describing the arrangement of molecules in a cell membrane.

Foreign antigen

An antigen not normally found in the body.

Founder effect

The reduction in genetic diversity that occurs when just a few organisms from a population start a new colony.

Frameshift mutation

A mutation that changes the number of bases in the DNA code, causing a shift in the base triplets that follow, so that the triplet code is read in a different way.

Functional RNA

RNA molecules that aren't mRNA, e.g. tRNA or the RNA found in ribosomes.

G

Gas exchange surface

A boundary between the outside environment and the internal environment of an organism, over which gas exchange occurs.

Gel electrophoresis

A technique that allows DNA fragments to be separated on a gel according to size.

Gene

A section of DNA that codes for a protein (polypeptide) which results in a characteristic.

Gene expression

The transcription of a gene into mRNA and translation of the mRNA into a protein.

Gene pool

The complete range of alleles present in a population.

Generator potential

The change in potential difference across a cell membrane due to the presence of a stimulus.

Gene technology

Techniques that allow the study and alteration of genes and their functions.

Gene therapy

Possible treatment option for genetic disorders and some cancers that involves altering defective genes inside cells.

Genetic bottleneck

An event that causes a big reduction in a population and reduces genetic diversity.

Genetic code

The sequence of base triplets (codons) in mRNA which code for specific amino acids.

Genetic disorder

An inherited disorder caused by an abnormal gene or chromosome.

Genetic diversity

The number of different alleles of genes in a species or population.

Genetic drift

The process whereby an allele becomes more common in a population due to chance.

Genetic engineering

See Recombinant DNA technology.

G

Gamete

A sex cell — e.g. the sperm cell in males or the egg cell in females.

Gas exchange

The process of taking in gases that are needed for life processes and getting rid of waste gases.

Genetic fingerprint

A DNA gel that shows the number of times repetitive, non-coding base sequences are repeated at different loci in an individual.

Genetic pedigree diagram

A diagram that shows how an inherited trait (characteristic) runs in a group of related individuals.

Genome

All the genetic material in an organism (or cell).

Genotype

The genetic constitution of an organism (the different alleles an organism has).

Geographical isolation

When a physical barrier, e.g. a flood, divides a population of a species, causing some individuals to become separated from the main population.

Germ line therapy

Gene therapy that involves altering the alleles in sex cells.

Gill

The respiratory organ of a fish.

Gill filament

A thin plate in a fish's gill.

Glomerular filtrate

The fluid present in the nephrons of the kidney, following ultrafiltration of the blood at the Bowman's capsule.

Glomerulus

A bundle of capillaries looped inside the Bowman's capsule of a nephron. Where ultrafiltration takes place.

Glucagon

A hormone secreted by the pancreas that has an important role in raising blood glucose concentration.

Gluconeogenesis

The conversion of glycerol or amino acids to glucose, activated by glucagon.

Glycogen

A polysaccharide made from a long, very branched chain of α -glucose.

Glycogenesis

The conversion of glucose to glycogen, activated by insulin.

Glycogenolysis

The conversion of glycogen to glucose, activated by glucagon.

Glycolipid

A lipid that has a carbohydrate attached.

Glycolysis

The first stage of aerobic respiration — here glucose is converted into pyruvate.

Glycoprotein

A protein that has a carbohydrate attached.

Glycosidic bond

A bond formed between monosaccharides.

Golgi apparatus

A group of fluid-filled flattened sacs. Involved with processing and packaging lipids and proteins, and making lysosomes.

Golgi vesicle

A small, fluid-filled sac produced by the Golgi apparatus, which stores and transports lipids and proteins.

Granum

A structure in chloroplasts formed from the stacking of thylakoid membranes.

Gravitropism (geotropism)

The growth of a plant in response to gravity.

Gross primary production (GPP)

The total amount of chemical energy converted from light energy by plants in a given area.

Guard cell

A cell that controls the opening and closing of stomata.

H**Habitat**

The place where an organism lives within an ecosystem.

Haemoglobin

An oxygen-carrying protein found in red blood cells.

Haploid

When a cell contains one copy of each chromosome.

Hardy-Weinberg principle

A mathematical model that predicts that the frequency of alleles in a population won't change from one generation to the next provided that certain conditions are met.

Helper T-cell

A T-cell that releases chemical signals to activate other immune system cells.

Herd immunity

Where unvaccinated people are protected because the occurrence of the disease is reduced by the number of people who are vaccinated.

Hereditary mutation

A mutation that's inherited from your parents.

Heterozygous

When an organism carries two different alleles at the same locus.

Histone

A protein that DNA wraps around to form chromatin, which makes up chromosomes.

HIV (human immunodeficiency virus)

A virus that affects the human immune system.

Homeostasis

The maintenance of a stable internal environment.

Homologous pair

A pair of matching chromosomes — each chromosome contains the same genes but could have different alleles.

Homozygous

When an organism carries two copies of the same allele at the same locus.

Host cell

A cell inside which a virus replicates or a cell that is used to carry recombinant DNA.

Humoral immune response

The immune response that involves B-cells, clonal selection and the production of antibodies.

Hydrolysis

A chemical reaction that uses a water molecule when it breaks bonds between molecules.

Hydrophilic

Attracts water.

Hydrophobic

Repels water.

Hydrostatic pressure

The pressure exerted by a liquid.

Hyperpolarisation

An increase in the potential difference across a cell's membrane, making it more negative than the resting potential.

Hypothalamus

A part of the brain that controls body temperature and monitors the water potential of the blood.

Hypothesis

A specific testable statement, based on a theory, about what will happen in a test situation.

I

Immunity

The ability to respond quickly to an infection.

Immunological comparison

Using antibodies to determine how similar proteins are.

Independent segregation

The random division of maternal and paternal chromosomes into daughter cells during meiosis.

Independent variable

The variable you change in an experiment.

Index of diversity

A measure of biodiversity that takes into account the number of species present in a community and the abundance of each species.

Indoleacetic acid (IAA)

An auxin produced in the tips of shoots and roots in flowering plants.

Inorganic ion

An ion (charged particle) that doesn't (usually) contain carbon.

Inspiration

Breathing in.

Insulin

A hormone secreted by the pancreas that has an important role in lowering blood glucose concentration.

Interphase

A period of the cell cycle in which the cell grows and DNA is replicated.

Interspecific competition

Competition between organisms of different species for the same resources.

Intraspecific competition

Competition between organisms of the same species for the same resources.

Intron

A section of DNA within a gene that does not code for amino acids.

In vitro cloning

When gene copies are made outside of a living organism using PCR.

In vivo cloning

When gene copies are made within a living organism as it grows and divides.

Iodine test

A biochemical test for the presence of starch.

iPS (induced pluripotent stem) cell

A type of pluripotent stem cell made in the lab by reprogramming a specialised adult body cell to express certain transcription factors.

K

Kinesis (kinetic response)

Non-directional (random) movement in response to a stimulus.

Krebs cycle

The third stage of aerobic respiration. It is a series of oxidation-reduction reactions that produces reduced coenzymes and ATP.

L

Lamella (in chloroplasts)

A thin, flat piece of thylakoid membrane found in chloroplasts.

Lamella (in fish)

A tiny structure found on the gill filament in a fish.

Leaching

The process in which water-soluble compounds in the soil are washed away, e.g. by rain.

Ligase

An enzyme that joins together the sticky ends of DNA fragments.

Light-dependent reaction

The first stage of photosynthesis. Light energy is absorbed by photosynthetic pigments and converted to ATP and reduced NADP.

Light-independent reaction (Calvin cycle)

The second stage of photosynthesis. Here ATP and reduced NADP (from the light-dependent reaction) are used to make glucose from carbon dioxide.

Limiting factor

A variable that can slow down the rate of a reaction.

Link reaction

The second stage of aerobic respiration where pyruvate is converted into acetyl coenzyme A.

Lipase

An enzyme that catalyses the hydrolysis of lipids.

Loading of oxygen (onto haemoglobin)

The action of an oxygen molecule binding with a haemoglobin molecule.

Locus

The position on a chromosome where a particular allele is found.

Loop of Henle

Part of the kidney nephron responsible for establishing the water potential gradient, which allows water to be reabsorbed by the kidney.

Lymphatic system

A network of tubes which transports excess tissue fluid back into the circulatory system.

Lysosome

A round organelle that contains digestive enzymes called lysozymes.

M

Magnification

How much bigger an image from a microscope is compared to the specimen.

Malignant tumour (cancer)

A tumour that invades and destroys surrounding tissues.

Margin of error

The range in which the true value of a measurement lies.

Marker gene

A gene that can be inserted into transformed cells in order to identify them.

Mark-release-recapture

A method used to estimate the population size of motile organisms.

Mass transport system

A system (e.g. the circulatory system) that carries substances to and from individual cells.

Mean

The average of the values collected in a sample, obtained by adding all the values together and dividing by the total number of values in the sample.

Medulla (oblongata)

A part of the brain that controls heart rate.

Meiosis

A type of cell division where a parent cell divides to create four genetically different haploid cells.

Memory cell

A white blood cell that remains in the body and remembers how to respond to infections.

Meristem

A growing region of a plant, e.g. the roots and shoots, which contains totipotent stem cells.

Mesophyll cell

A type of plant cell present in a leaf and the main gas exchange surface in a plant.

Metabolic rate

The rate at which energy is used by an organism.

Methylation

Attachment of a methyl group to something (e.g. DNA).

Micelle (digestion)

A microscopic structure composed of monoglycerides, fatty acids and bile salts.

Microarray

A glass slide with microscopic spots of different DNA probes attached to it in rows.

Microfibril

A strong fibre formed by chains of cellulose linked together by hydrogen bonds.

Microvillus

A fold in the cell-surface membrane that increases the surface area.

miRNA

Small, single-stranded RNA molecules that can interfere with the translation of genes.

Mitochondrion

An oval-shaped organelle with a double membrane. The site of anaerobic respiration.

Mitosis

A type of cell division where a parent cell divides to produce two genetically identical daughter cells.

Monoclonal antibody

An antibody produced from a single group of genetically identical B-cells.

Monohybrid inheritance

The inheritance of a single characteristic (gene) controlled by different alleles.

Monomer

A small, basic molecular unit, e.g. amino acids and monosaccharides.

Monosaccharide

A monomer of carbohydrates.

mRNA (messenger RNA)

A type of RNA that is the template for protein synthesis. It carries the genetic code from the DNA in the nucleus into the cytoplasm.

Multicellular organism

An organism that has more than one cell, e.g. a human.

Multiple repeat

A section of repetitive DNA found outside of genes — does not code for amino acids.

Multipotent stem cell

A stem cell only able to develop into a few types of cell.

Mutagenic agent

Something that increases the rate of DNA mutations.

Mutation

A change in the base sequence of an organism's DNA.

Mycorrhiza

A symbiotic relationship between a fungus and the roots of a plant.

Myelin sheath

A layer of Schwann cells around a neurone that acts as an electrical insulator and speeds up conduction of nervous impulses.

Myocardial infarction

A heart attack.

Myofibril

A long, cylindrical organelle within a muscle fibre that's highly specialised for contraction.

Myogenic contraction

When muscle cells are able to contract and relax without receiving signals from nerves, e.g. heart cells.

Myosin

The protein that makes up the thick myofilaments in myofibrils.

N

NAD

A type of coenzyme involved in respiration. It transfers hydrogen from one molecule to another.

NADP

A coenzyme involved in photosynthesis. It transfers hydrogen from one molecule to another.

Natural selection

The process whereby an allele becomes common in a population because it codes for a characteristic that makes an organism more likely to survive, reproduce and pass on its genes to its offspring.

Negative control

An extra experiment set up to check that only the independent variable is affecting the dependent variable. It is not expected to have any effect.

Negative feedback mechanism
A mechanism that restores a level back to normal in a system.

Nephron
One of the filtering units of the kidney, responsible for removing waste products from the blood and involved in controlling the water potential of the blood.

Net primary production (NPP)
The energy available to plants for growth and reproduction (after respiratory loss has been deducted from GPP) and the energy available to the next trophic level in a food chain.

Net production
The energy in consumers that is available to the next trophic level in a food chain.

Neuromuscular junction
A specialised cholinergic synapse between a motor neurone and a muscle cell.

Neurotransmitter
A chemical that transmits a nerve impulse across a synapse.

Niche
The role of a species within its habitat, e.g. what it eats, and where and when it feeds.

Nitrification
The process in which ammonia and ammonium ions in the soil are changed into nitrogen compounds by nitrifying bacteria.

Nitrogen fixation
The process in which nitrogen gas in the atmosphere is turned into nitrogen-containing compounds, such as ammonia.

Node of Ranvier
A tiny area of bare cell membrane on the surface of a myelinated neurone, where depolarisation can take place.

Non-competitive inhibitor
A molecule that binds away from an enzyme's active site and alters the shape of the active site, so the substrate can no longer bind.

Non-reducing sugars
A class of monosaccharides and disaccharides.

Normal distribution
A bell-shaped curve symmetrical about the mean.

Nucleic acid
A polymer made from nucleotides, e.g. DNA and RNA.

Nucleolus

A structure within a nucleus that makes ribosomes.

Nucleotide
The monomer that makes up polynucleotides — consists of a pentose sugar, a phosphate group and a nitrogenous organic base.

Nucleus
An organelle that contains chromosomes and controls a eukaryotic cell's activities.

Null hypothesis
A hypothesis that states there's no difference or correlation between the factors being investigated.

O

Oestrogen
A steroid hormone released by the ovaries that stimulates the uterus lining to thicken in the menstrual cycle.

Oncogene
A mutated proto-oncogene that stimulates cells to divide uncontrollably.

Organ
A group of different tissues that work together to perform a particular function.

Organ system
A group of organs that work together to carry out a particular function.

Organelle
A part of a cell, e.g. the nucleus.

Osmoreceptor
A cell in the hypothalamus which monitors the water potential of the blood.

Osmoregulation
The regulation of the water potential of the blood.

Osmosis

Diffusion of water molecules across a partially permeable membrane, from an area of higher water potential to an area of lower water potential.

Oxidation

A chemical reaction where a molecule loses electrons, and may have lost hydrogen or gained oxygen.

Oxidative phosphorylation

The final stage in aerobic respiration. Energy carried by electrons, from reduced coenzymes, is used to make ATP.

Oxygen dissociation curve

A curve on a graph that shows how saturated with oxygen haemoglobin is at any given partial pressure.

Oxyhaemoglobin

The molecule formed when oxygen binds to haemoglobin.

P

Pacinian corpuscle

A type of receptor found in your skin which detects mechanical stimuli.

Palindromic sequence

A sequence of DNA bases that consists of antiparallel base pairs (base pairs that read the same in opposite directions).

Partially permeable membrane

A membrane that lets some molecules through it, but not others.

Pathogen

An organism that causes disease.

pCO₂

Partial pressure of carbon dioxide — a measure of carbon dioxide concentration.

PCR (polymerase chain reaction)

A technique used to make millions of identical copies of a DNA fragment in a few hours.

Peptidase

An enzyme that catalyses the hydrolysis of proteins.

Peptide bond

A bond formed between amino acids.

| | | |
|--|---|---|
| Phagocyte | Plagioclimax | Positive control |
| A type of white blood cell that carries out phagocytosis, e.g. a macrophage. | The climax community produced when succession is artificially stopped by human activities. | An extra experiment set up to check what a positive result looks like. |
| Phagocytosis | Plasma cell | Positive feedback mechanism |
| The engulfment of pathogens. | A type of B-cell that produces antibodies. | A mechanism that amplifies a change away from the normal level in a system. |
| Phenotype | Plasma membrane | Posterior pituitary gland |
| The expression of the genetic constitution of an organism and its interaction with the environment (what characteristics an organism has as a result of its genes and the effect the environment has on them). | See cell-surface membrane. | Part of the pituitary gland (a gland located at the base of the brain), which secretes hormones such as antidiuretic hormone (ADH). |
| Phenotypic ratio | Plasmodesma | Potential difference |
| The ratio of different phenotypes (characteristics) in the offspring of a genetic cross. | A small channel in a plant cell wall that connects neighbouring plant cells. | The voltage across a cell membrane. |
| Phloem | Pluripotent stem cell | Potometer |
| A tissue in plants that transports organic substances (e.g. sucrose) from their source to their sink. | A stem cell that can develop into any type of cell, apart from cells that make up the placenta. | A special piece of apparatus used to estimate transpiration rates. |
| Phospholipid | pO₂ | Precise result |
| A lipid containing one molecule of glycerol attached to two fatty acids and a phosphate group. Main component of the cell membrane. | Partial pressure of oxygen — a measure of oxygen concentration. | A result that is close to the mean. |
| Phosphorylation | Polymer | Predation |
| The process of adding a phosphate group to a molecule. | A large, complex molecule composed of long chains of monomers, e.g. proteins and carbohydrates. | Where an organism (the predator) kills and eats another organism (the prey). |
| Photoionisation | Polynucleotide | Prediction |
| The process of turning an atom or a molecule into an ion using light energy. | A molecule made up of lots of nucleotides joined together in a long chain. | See hypothesis. |
| Photolysis | Polypeptide | pre-mRNA |
| The splitting (lysis) of a molecule using light (photo) energy. | A molecule formed from more than two amino acids. | mRNA in eukaryotes containing both introns and exons. It is spliced to form mRNA. |
| Photophosphorylation | Polysaccharide | Primary immune response |
| The process of adding phosphate to a molecule using light energy. | A molecule formed from more than two monosaccharides. | The immune response triggered when a foreign antigen enters the body for the first time. |
| Photoreceptor | Population | Primary succession |
| A receptor in the eye that detects light. | All the organisms of one species in a habitat. | Succession that happens on newly formed or exposed land with no soil. |
| Phototropism | Population growth curve | Primer |
| The growth of a plant in response to light. | A graph showing the change in the size of a population over time. | A short piece of single stranded DNA that is complementary to the bases at the start of the DNA fragment you want to copy. |
| Phylogeny | Population growth rate | Prokaryote |
| The evolutionary history of groups of organisms. | How much the size of a population increases or decreases in a year. | Single-celled organism without a nucleus or membrane-bound organelles, e.g. bacteria. |
| Pioneer species | Population sample | Promoter region |
| The first species to colonise an area during succession. | A small group of organisms used as a model for the whole population. | A DNA sequence that tells the enzyme RNA polymerase where to start producing mRNA. |
| | | Proteome |
| | | The full range of proteins an organism (or cell) is able to produce. |

Proto-oncogene

A gene that produces proteins that make cells divide.

Purkyne tissue

Fine muscle fibres in the heart that carry waves of electrical activity into the muscular walls of the right and left ventricles.

Q**Quadrat**

A square frame, usually divided into 100 smaller squares, used for investigating populations of non-motile or slow-moving species.

Qualitative test

A qualitative test tells you what's present, e.g. an acid or an alkali.

Quantitative test

A quantitative test tells you how much of something is present, e.g. an acid that's pH 2.46.

R**Random error**

A difference in a measurement caused by an unpredictable factor, e.g. human error.

Receptor

A cell, or protein on a cell surface membrane, that detects a stimulus.

Recessive allele

An allele whose characteristic only appears in the phenotype if there are two copies present.

Recognition sequence

A specific palindromic sequence in DNA recognised by a restriction endonuclease.

Recombinant DNA

The name for DNA formed by joining together DNA from different organisms.

Recombinant DNA technology

When DNA from different organisms is joined together by isolating a fragment from a donor organism and inserting it into the DNA of a host organism. Also known as genetic engineering.

Reducing sugars

A class of monosaccharides and disaccharides.

Reduction

A chemical reaction where a molecule gains electrons, and may have gained hydrogen or lost oxygen.

Reflex

A rapid, involuntary response to a stimulus.

Refractory period

The period following an action potential in which a neurone cell membrane can't be excited.

Repeatable result

A result than can be repeated by the same person using the same method and equipment.

Repolarisation

The return of a cell membrane to its resting potential.

Repressor

A transcription factor that inhibits or decreases the rate of transcription.

Reproducible result

A result that can be consistently reproduced in an independent experiment.

Reproductive isolation

When changes in allele frequency mean that some individuals of the same species can no longer interbreed to produce fertile offspring.

Resolution

How well a microscope distinguishes between two points close together.

Respiratory loss

The amount of energy lost to the environment as heat when organisms respire.

Resting potential

The potential difference across a cell membrane when the cell is at rest.

Restriction endonuclease enzyme

An enzyme that recognises specific recognition sequences and cuts DNA at these places.

Restriction map

A diagram of a piece of DNA showing where different recognition sites of restriction enzymes are found.

Retina

The part of the eye containing photoreceptor cells, which light is focussed onto.

Reverse transcriptase

An enzyme that makes a DNA copy of RNA.

Ribosome

An organelle found in the cell cytoplasm that assembles proteins.

Ribulose bisphosphate carboxylase (rubisco)

An enzyme which catalyses the formation of glycerate 3-phosphate from carbon dioxide and ribulose bisphosphate (RuBP) in the light-independent reaction of photosynthesis.

Risk factor

Anything that increases the chance of getting a disease.

RNA (ribonucleic acid)

A type of nucleic acid, similar to DNA but containing ribose instead of deoxyribose sugar and uracil instead of thymine.

RNA interference

The mechanism by which siRNA or miRNA affects translation.

RNA polymerase

An enzyme that synthesises RNA from DNA.

Rod (eye)

A photoreceptor cell found in the eye that gives information in black and white.

S**Saltatory conduction**

The process in myelinated neurones by which a nervous impulse travels between nodes of Ranvier.

Sample size

The number of samples in the investigation, e.g. the number of people in a drug trial.

Saprobiont

A microorganism that feeds on the remains of dead plants and animals, using extracellular digestion to break down the remains.

Saprobio
nutritio

Obtaining nutrients from dead organic matter using extracellular digestion.

Sarcomere

A short contractile unit that's part of a myofibril, made up of overlapping myosin and actin filaments.

Sarcoplasmic reticulum

A network of internal membranes that runs through the sarcoplasm. It stores and releases calcium ions that are needed for muscle contraction.

Saturated fatty acid

A fatty acid with no double bonds between its carbon atoms.

Schwann cell

The type of cell that makes up the myelin sheath around neurones.

Secondary immune response

The immune response triggered when a foreign antigen enters the body for the second time.

Secondary succession

Succession that happens on land cleared of all plants but where the soil remains, e.g. after a forest fire.

Second messenger

A chemical that's produced inside a cell in response to a signal outside the cell. The chemical relays the signal to the inside of the cell.

Selective reabsorption (kidneys)

The reabsorption of useful substances along the kidney nephron back into the blood.

Semi-conservative replication of DNA

Replication of DNA in which half of the new molecules of DNA are from the original piece of DNA.

Semi-lunar (SL) valve

A valve in the heart linking the ventricles to the aorta and pulmonary artery.

Serial dilution

The creation of a set of solutions that decrease in concentration by the same factor each time.

Sex-linked characteristic

When the allele that codes for the characteristic is located on a sex chromosome (X or Y).

Sink (translocation)

A part of a plant where substances (e.g. sucrose and amino acids) are used up.

Sino-atrial node (SAN)

A group of cells in the wall of the right atrium that set the rhythm of the heartbeat by sending out regular waves of electrical activity to the atrial walls.

siRNA (small interfering RNA)

A double-stranded RNA molecule that can interfere with the transcription and translation of genes.

Sister chromatid

One of two identical copies of a chromosome joined together in the middle.

Sliding filament theory

The theory that myosin and actin filaments slide over one another to make sarcomeres contract.

Slow twitch muscle fibre

A muscle fibre that contracts slowly and can work for a long time without getting tired.

Somatic gene therapy

Gene therapy that involves altering the alleles in body cells.

Source (translocation)

A part of a plant where substances needed by the plant (e.g. sucrose and amino acids) are produced.

Specialised cell

A cell adapted to carry out specific functions.

Speciation

The development of a new species from an existing species.

Species

A group of similar organisms that can reproduce to give fertile offspring.

Species richness

The number of different species in a community. A measure of biodiversity.

Specific base pairing

See complementary base pairing.

Spiracle

A pore on the surface of an insect.

Splicing

The process by which introns are removed from pre-mRNA strands and exons are joined to form mRNA.

Stabilising selection

Where individuals with alleles for characteristics towards the middle of the range are more likely to survive, reproduce and pass on their alleles.

Standard deviation

A measure of the spread of values about the mean.

Starch

A carbohydrate molecule made up of two polysaccharides — amylose and amylopectin.

Stem cell

An unspecialised cell that can develop into other types of cell. It's also able to divide to form new cells.

Stem cell therapy

Using stem cells to treat or cure medical disorders.

Sticky end

A small tail of unpaired DNA bases at the end of a DNA fragment.

Stimulus

A change in an organism's internal or external environment.

Stoma

A pore in the epidermis of a plant leaf.

Stroke volume

The volume of blood pumped during each heartbeat (measured in cm³).

Stroma

A thick fluid found in chloroplasts.

Substrate

A substance that interacts with an enzyme.

Succession

The process by which an ecosystem changes over time.

Sugar-phosphate backbone

Alternating sugar and phosphate groups joined together in a polynucleotide chain.

Summation

The process in which the effect of a neurotransmitter released from many neurones (or one neurone that's stimulated a lot in a short period of time) is added together.

Supercoiling

The way that DNA is condensed to fit in the cell in prokaryotes.

Surface area:volume ratio

An organism or structure's surface area in relation to its volume.

Survival curve

A graph which shows the percentage of all the individuals that were born in a population that are still alive at any given age.

Sympatric speciation

Where speciation occurs without populations of a species being geographically isolated.

Synapse

A junction between a neurone and another neurone, or between a neurone and an effector cell.

T

Target cell

A cell that has specific receptors for a particular type of chemical, such as a hormone or a neurotransmitter.

Taxis (tactic response)

Directional movement in response to a stimulus.

Taxon

A group within a classification hierarchy, e.g. domain, kingdom.

T-cell

A type of white blood cell involved in the immune response. Some types activate B-cells and some kill pathogens directly.

Temporary mount

A method of preparing a microscope slide in which the specimen is suspended in a drop of liquid.

Terminator region

A DNA sequence that tells the enzyme RNA polymerase where to stop producing mRNA.

Theory

A possible explanation for something.

Thylakoid membrane

A membrane found inside chloroplasts, stacked up to form grana.

Tissue

A group of similar cells working together to perform a particular function.

Tissue fluid

The fluid that surrounds cells in tissues.

Totipotent stem cell

A stem cell able to develop into any type of body cell.

Toxin

A harmful molecule. Released by some pathogens.

Trachea (insects)

A pipe that carries air between the external environment and the inside of an insect's body.

Tracheole

A small pipe that branches off the trachea in an insect and is used for gas exchange.

Transcription

The first stage of protein synthesis, in which an mRNA copy of a gene is made from DNA.

Transcription factor

A protein molecule that controls the transcription of a gene.

Transect

A line used to help find out how non-motile or slow-moving organisms are distributed across an area, e.g. how species change from a hedge towards the middle of a field.

Transformed cell

A host cell that has taken up recombinant DNA.

Transformed organism

A plant, animal or microorganism that has had its genes altered by recombinant DNA technology.

Translation

The second stage of protein synthesis, in which amino acids are joined together by ribosomes to make a polypeptide chain (protein).

Translocation

The movement of solutes to where they're needed in a plant.

Transpiration

The evaporation of water from a plant's surface.

Triglyceride

A lipid containing one molecule of glycerol attached to three fatty acids.

Triplet

A series of three bases which codes for one amino acid in a protein.

tRNA (transfer RNA)

A type of RNA involved in translation. It carries the amino acids used to make proteins to the ribosomes.

Trophic level

A stage in a food chain.

Tropism

The response of a plant to a directional stimulus.

Tropomyosin

A protein found between actin filaments attached to another protein. Together the two proteins help myofilaments move past each other.

Tumour

A mass of abnormal cells.

Tumour suppressor gene

A gene that slows the rate of cell division by producing proteins that stop cells dividing or cause them to self-destruct.

U

Ultracentrifugation

A method where cell components are separated out using a centrifuge.

Ultrafiltration (kidneys)

The filtering of the blood that takes place under high pressure, as blood passes from the glomerulus into the Bowman's capsule.

Uncertainty (in data)

The amount of error measurements might have.

Unipotent stem cell

A stem cell that can only differentiate into one type of cell.

Unloading of oxygen (from haemoglobin)

The action of an oxygen molecule being released from a haemoglobin molecule.

Unsaturated fatty acid

A fatty acid with at least one double bond between its carbon atoms.

X

Xerophyte

A plant specially adapted for life in a warm, dry or windy habitat.

Xylem

A tissue in plants that transports water and mineral ions up a plant from the roots to the leaves.

V

Vaccination

The administering of a vaccine containing antigens to give immunity.

Vacuole

An organelle that contains cell sap (a weak solution of sugar and salts).

Valid result

A result that answers the original question and for which all the variables that could have affected it were controlled.

Variable

A quantity that has the potential to change, e.g. weight, temperature, concentration.

Variation

The differences that exist between individuals.

Vector (in gene technology)

Something used to transfer DNA into a cell, e.g. plasmids or bacteriophages.

Ventilation

Breathing in and breathing out.

Virus

An acellular structure that invades and reproduces inside the cells of other organisms (causing disease).

Visual acuity

The ability to tell apart points that are close together.

Z

Zygote

The diploid cell formed when two gametes fuse during fertilisation.

W

Water potential

The likelihood of water molecules to diffuse into or out of a solution.

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Data acknowledgements

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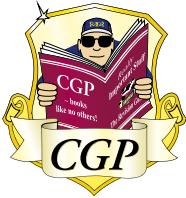
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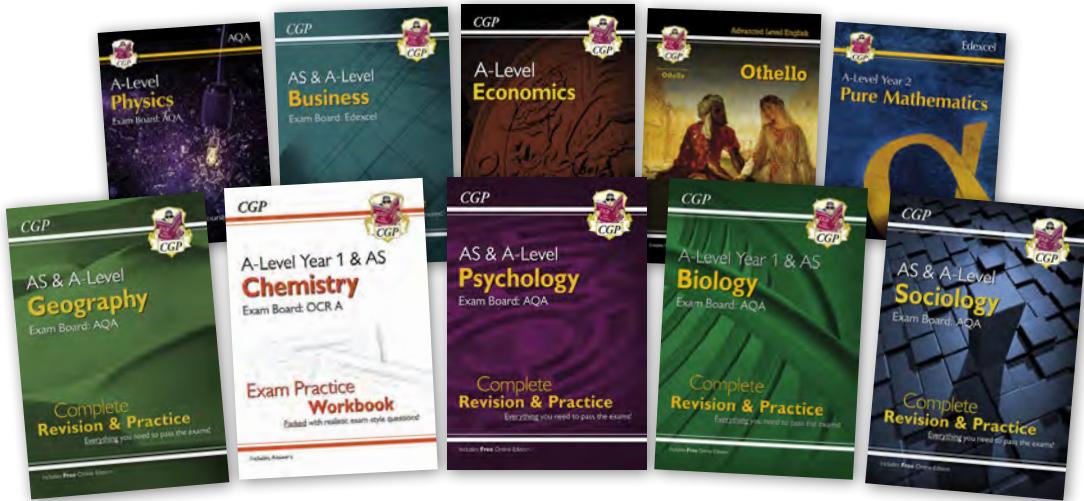
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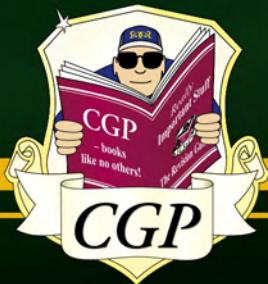
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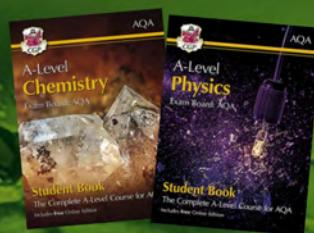
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